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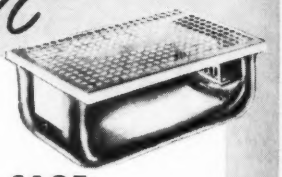
Fiftieth Anniversary
1906 - 1956

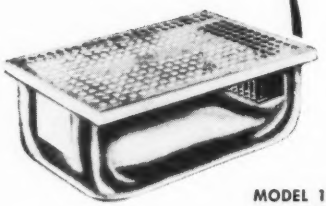
Published quarterly by the
FEDERATION OF AMERICAN SOCIETIES
for EXPERIMENTAL BIOLOGY

Leading Laboratories *Prefer* MOUSE-HOUSE



THE *Plastic* ANIMAL CAGE

because..



1. **SPEEDS RESEARCH**—clear, plastic walls permit daily cage inspection, aid observation of rodent development. False mesh bottoms speed metabolism tests and keep animals clean.

2. **CLEANER** — lower portion of cage is free from crevices and corners, thus cleaning usually involves only (1) turning cage upside down and (2) brushing unclean surfaces with hot soap suds and rinsing in hot water. Body acids and urine do not affect plastic cages. We recommend our HYTRON as a disinfectant.

3. **SAFER** — slow changing temperatures of smooth plastic walls protect rodents against drafts, and sudden temperature changes, thus reducing mortality rate.

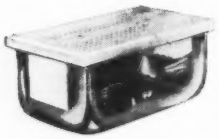
4. **VERMIN PROOF** — the plastic MOUSE-HOUSE automatically eliminates the outside enemies to highly technical research.

5. **LESS COSTLY** — by comparison with utility value of other cages. With reasonable handling these cages last indefinitely, making them more economical.

MODEL 150
STANDARD

CLEAR PLASTIC CAGE FOR RATS AND HAMSTERS. Molded from crystal clear plastic sheets; interchangeable stainless steel perforated cover fits snugly on the fluted sides of cage and has two special openings for inserting drinking tubes. Stainless steel feed cup fits over the end of the cage, inside the top rim, has a slotted base and a suspended hood. Permanent record card on end. Cage size for all 150 Models 11½" x 18½" x 6½".

Follow the lead of a large majority of research departments in leading universities, hospitals, privately endowed institutions and government bureaus who are regular users of this modern plastic animal cage. Everyone who uses this practical economical way of caging experimental rodents say the MOUSE-HOUSE cage is superior in every way.

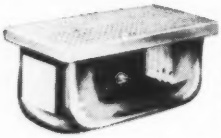


MODEL 101

CLEAR PLASTIC STOCK CAGE FOR MICE. Has all the construction features described for Model 150, but is smaller. Accommodates small rodents, cage size 11½" x 7½" x 5".

CLEAR PLASTIC CAGE FOR RATS AND HAMSTERS. Has same features of other plastic cages plus a stainless perforated slide-on cover with built-in food hopper which can be filled without opening the cage, plus a removable expanded metal grate above the plastic bottom. Grate is removable for cleaning. Size 13" x 9½" x 7".

MODEL 110

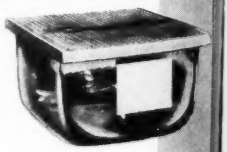


MODEL 103 STANDARD

CLEAR PLASTIC CAGE FOR MICE. This cage has all the construction features described for Model 101 except the size is smaller. Cage size for all 103 Models 11⅞" x 5⅛" x 5".

MODEL 102 STANDARD

CLEAR PLASTIC CAGE FOR MICE. Has all the construction features described for Model 101 except the general shape is square instead of rectangular. Cage size for all 102 Models 11¼" x 11¼" x 6½".



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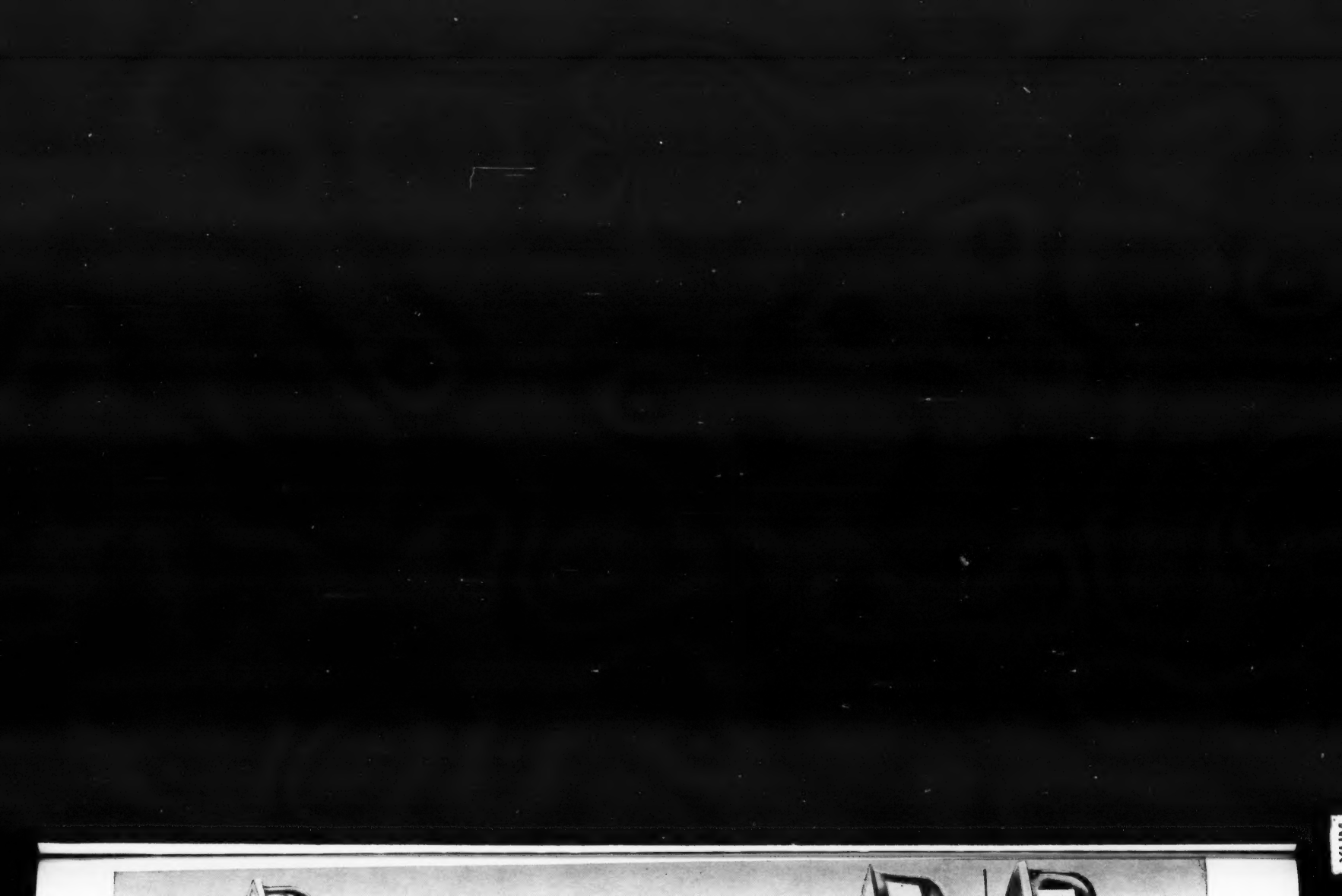
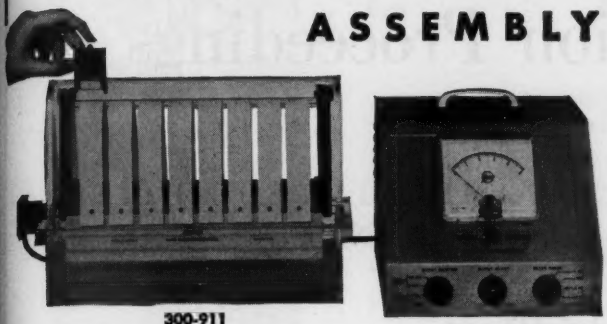


Spinco Model R

PAPER ELECTROPHORESIS ASSEMBLY



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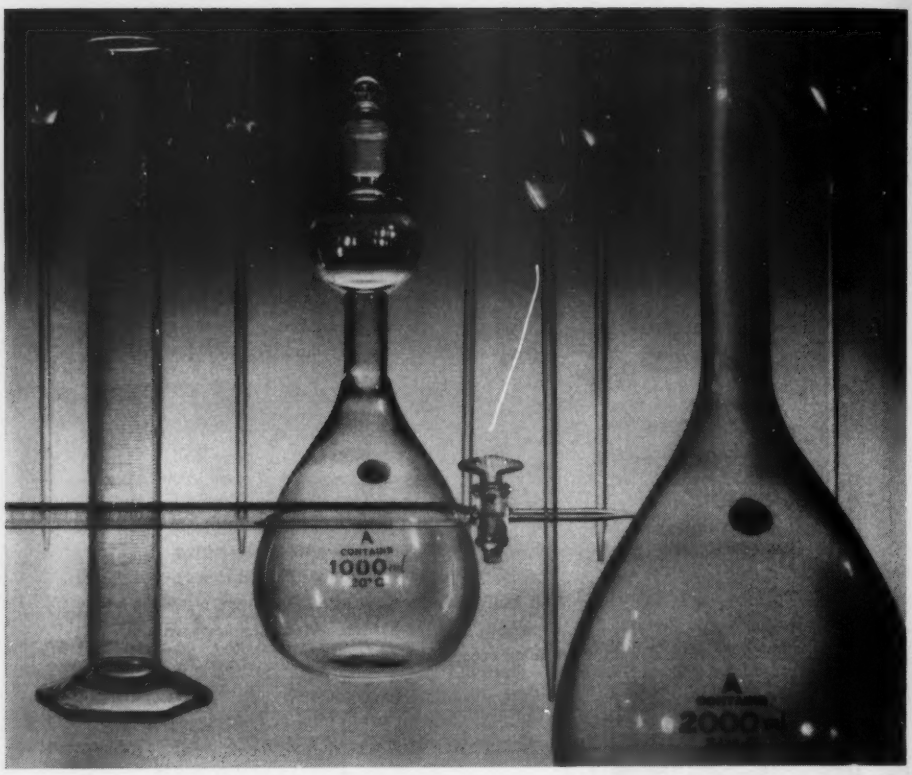
*The American Foundation: Medical Research: A Midcentury Survey, Boston, Little, Brown and Company, 1955, vol. 1, p. XXXI.

**Ibid., p. 600.



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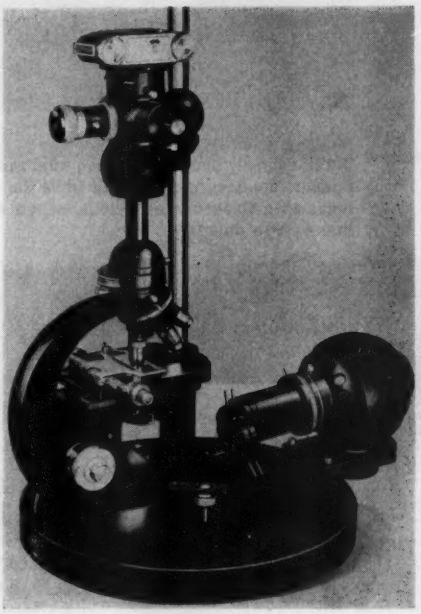

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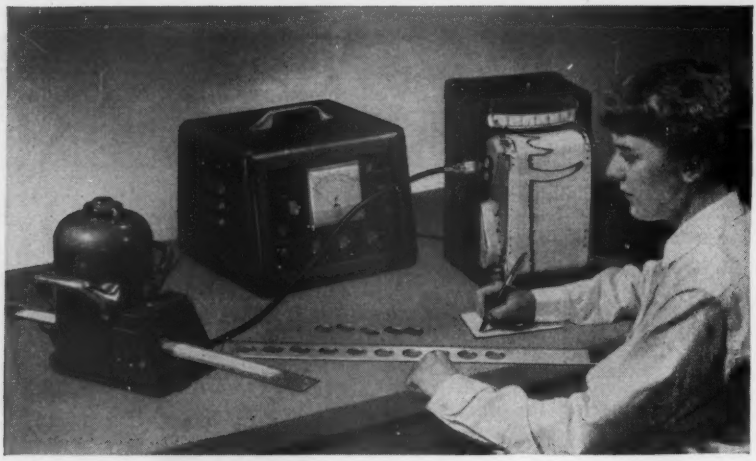

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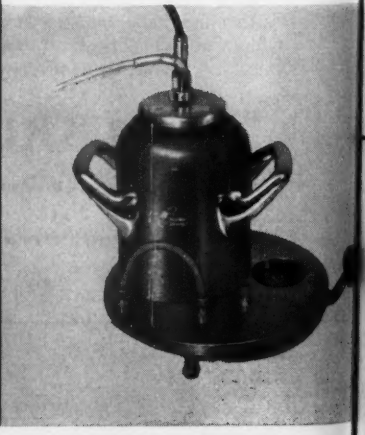

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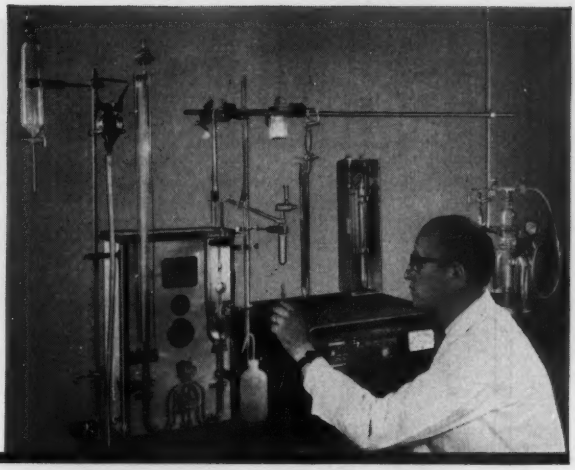

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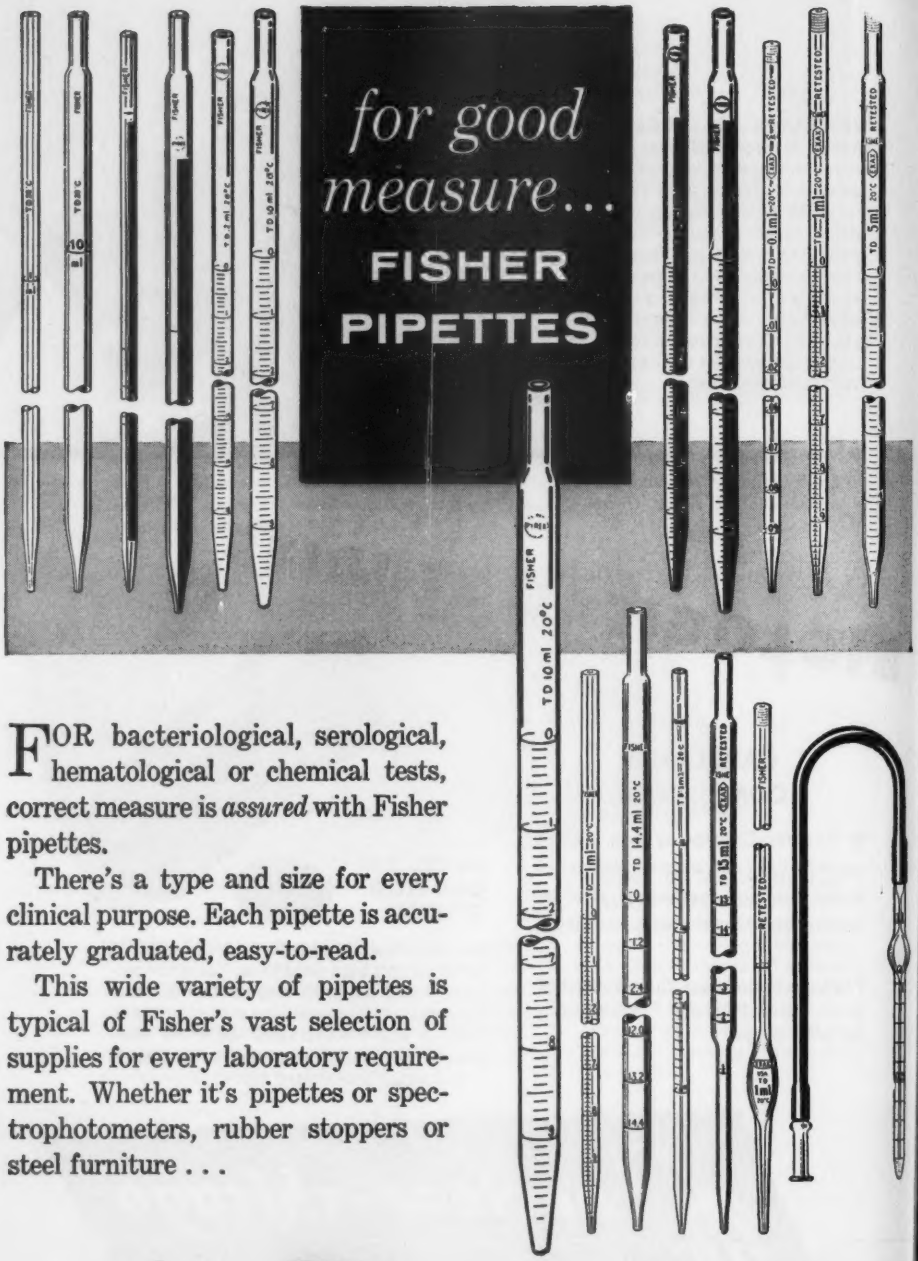

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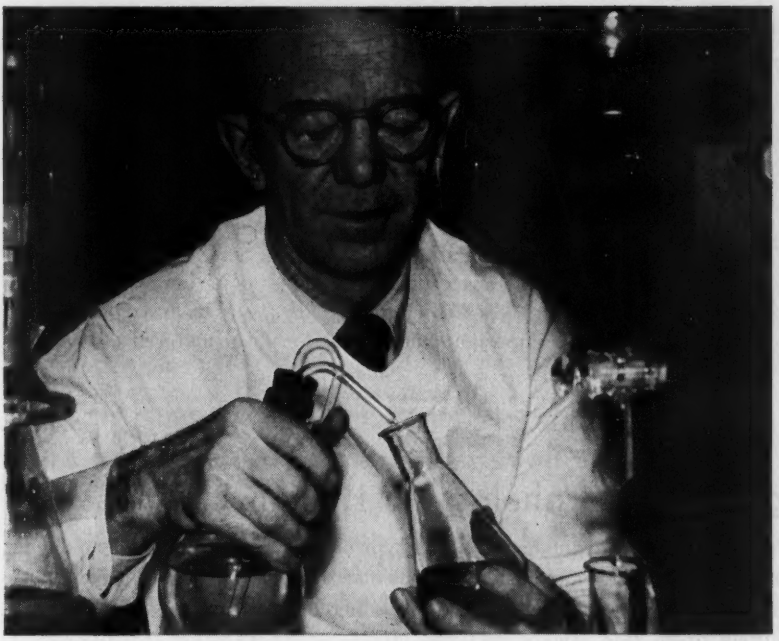

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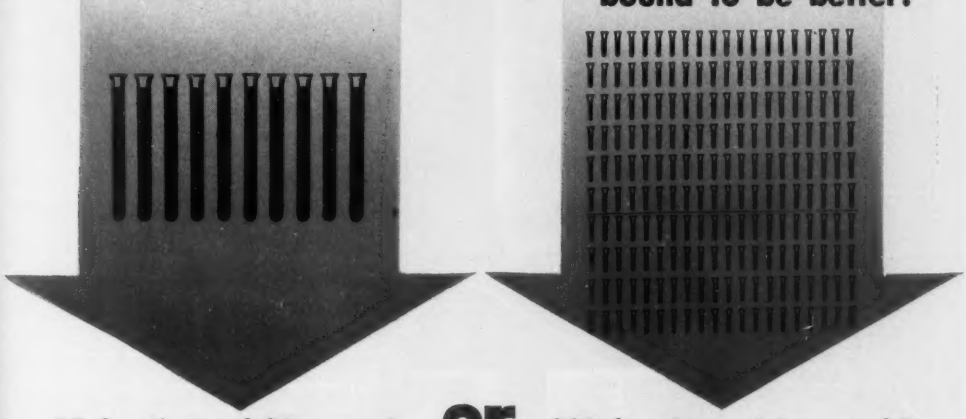

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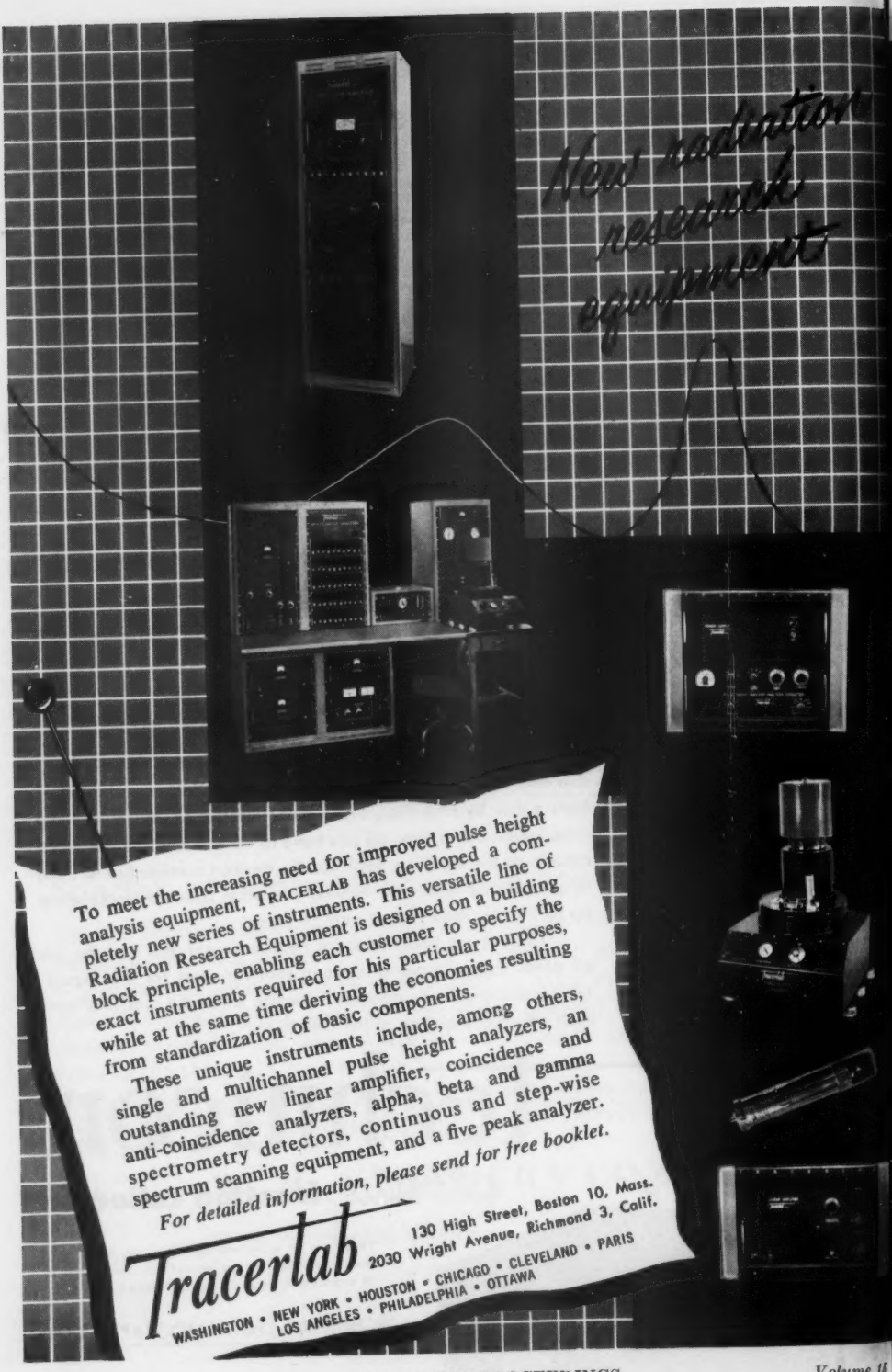
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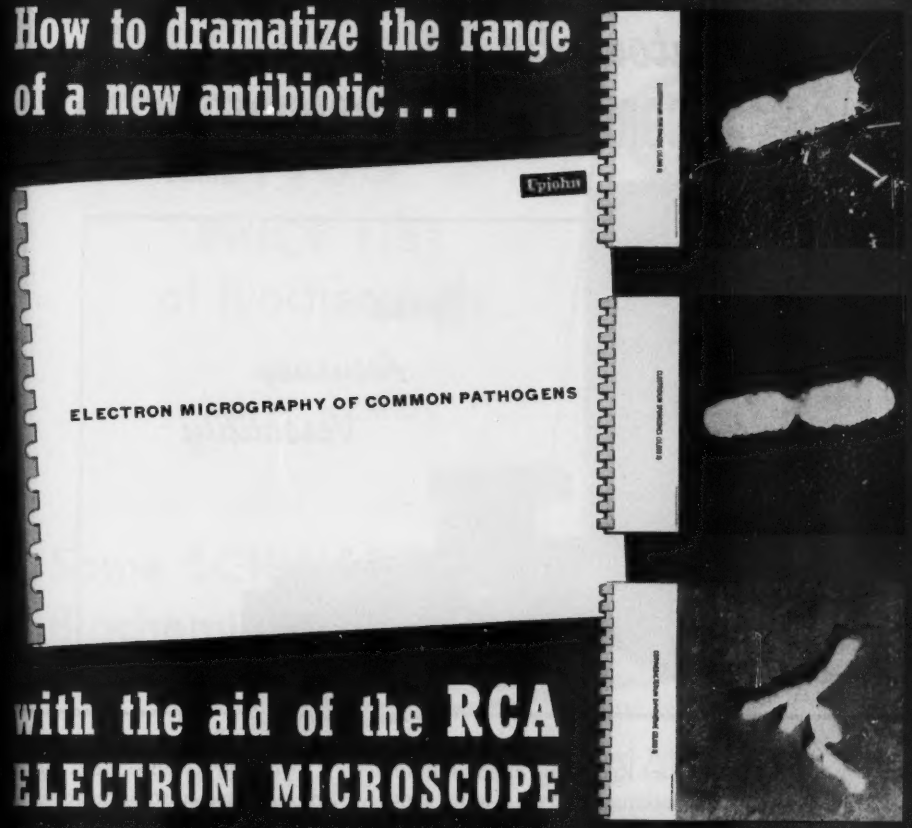

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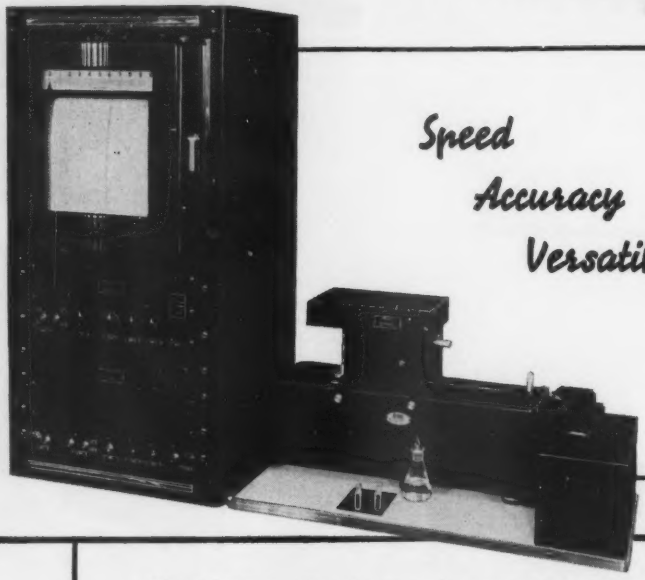

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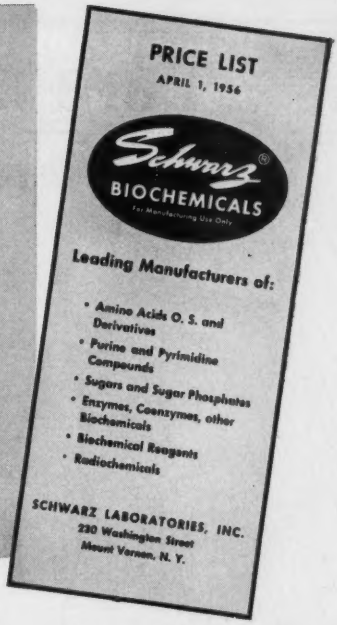

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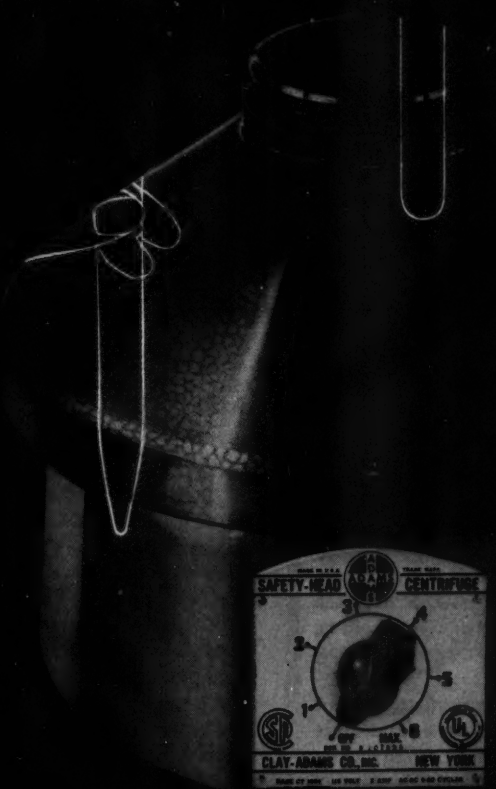

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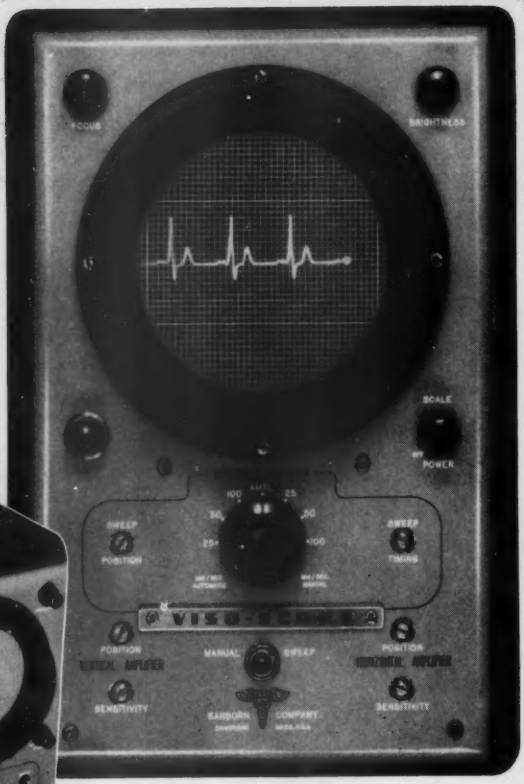


MODEL 169A

SANBORN VISO-SCOPE

DESIGNED for compatibility with other SANBORN equipment (examples, right), simplified operation via minimum controls (illustration, above), and *precise* control of the base line sweep, the Model 169A Viso-Scope now permits the owner or purchaser of SANBORN recording instruments to greatly widen their field of usefulness.

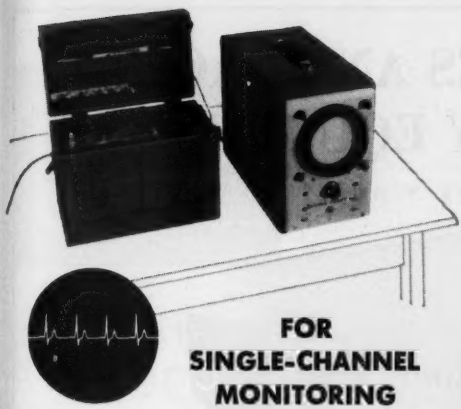
The cathode ray tube selected for this 'scope has a *dual coating* which produces a beam image of exceptional sharpness and long persistence on the orange screen-filter (when monitoring) and of extreme brightness and short duration on the blue screen-filter (for photography). When photography is desired, a Fairchild or DuMont oscilloscope camera may be mounted on the screen bezel (or any common camera may be used).

Sweep speeds of 25, 50 and 100 millimeters per second are obtainable instantaneously, and enlargement on the Viso-Scope screen of a segment of the tracing which is of particular interest may be made at any time.

Typical applications of the Viso-Scope include: observation of changes in the electrocardiogram resulting from use of drugs; pressure monitoring in catheterization during manipulation of the catheter; and a more precise study of complex wave forms by an instantaneous increasing of the sweep speed, such as in myography, phonocardiography, and small animal electrocardiography.

Descriptive literature on the Viso-Scope complete with diagrams showing hook-up requirements of various Sanborn recorders is available on request.

The Model 169A-OR (Operating Room) Viso-Scope is a completely self-contained oscilloscope-amplifier unit, specially designed for visual ECG presentation during surgery in the presence of explosive gases. Viewing unit is supported at or above five foot height on a steel column, and may be turned and tilted for best viewing angle. Provision is made for connection to a remote ECG, when written records are also desired. May also be used for display of sphygmograms, pneumograms, etc.



FOR SINGLE-CHANNEL MONITORING

Electrocardiograms may be continuously monitored with a Viso-Scope connected directly to a Sanborn Viso-Cardiette (Model 572 or 51). Or, a Model 128 or 141 Sanborn Recorder may be similarly used for viewing other phenomena such as pressure, temperature, sphygmograms, pneumograms, myograms, etc., with suitable transducers employed.

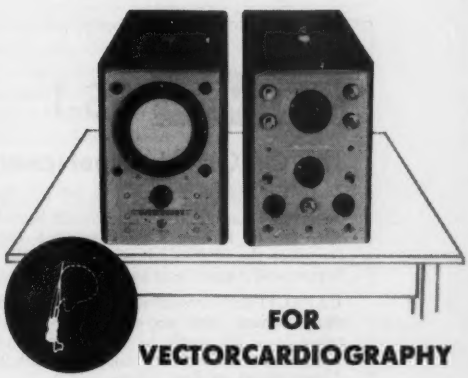
Written records may be made whenever desired during the monitoring. Selection of sweep speeds on the Viso-Scope (see left page) is particularly advantageous to owners of these instruments.

FOR MONITORING UP TO FOUR EVENTS SIMULTANEOUSLY

The Sanborn Electronic Switch, shown above connected to each channel of a Model 154M four-channel Poly-Viso, is designed for use with any Sanborn two- or four-channel *direct writing* recording system. It permits *simultaneous* observation of as many phenomena as the system is set up to record, and Viso-Scope thus connected appears to be operating with *separate beams*. Controls permit liberal relative positioning of beams on the scope screen, and *individual* sensitivity control. These adjustments have no effect on the recording instrument.

HIGHER SPEED MONITORING

With the addition of an attachable booster amplifier the Viso-Scope may be used with a Sanborn Twin-Beam Cardiette, to display phenomena of higher frequency such as are encountered in phonocardiography, small animal electrocardiography, high fidelity human electrocardiography, etc. Separate oscilloscope jacks on the Twin-Beam permit ready selection of the signal from either "phono" or ECG channel, for alternate display on the Viso-Scope screen.



FOR VECTORCARDIOGRAPHY

The Sanborn Model 185 Vector System (or vector amplifier) shown above right, has been specifically designed for use with the Viso-Scope, for displaying either vector loops or ECG complexes. This instrument's lead selector switch, together with a specially-marked 9-wire patient cable and extra electrodes, permit the instantaneous selection of either cube or tetrahedral vector leads, or ECG leads, for Viso-Scope display. Adequate sensitivity (10" per mv) provides clear showing of P and T loops, as well as QRS. Additionally (via installation of timing transformer in the Viso-Scope) the Vector system provides Z axis, or intensity modulation for indication of speed and direction of sweep trace.

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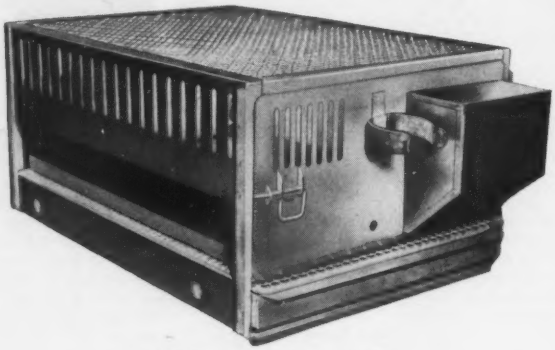

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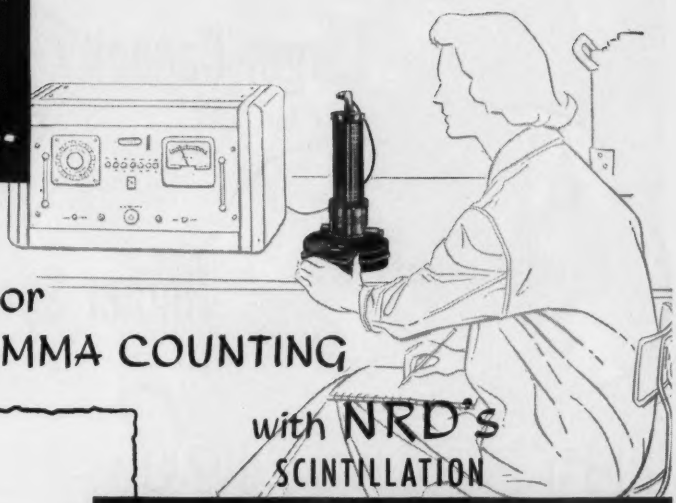

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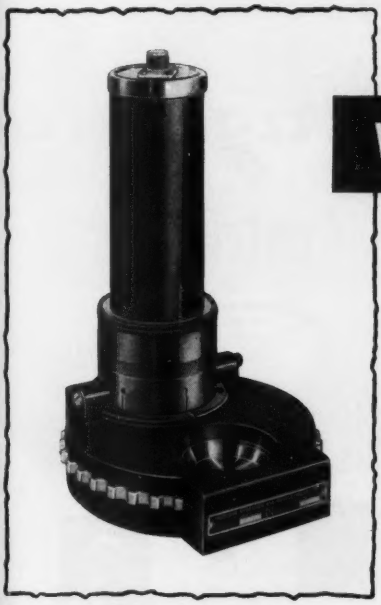

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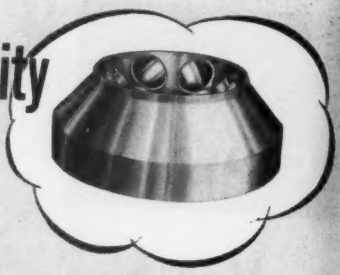

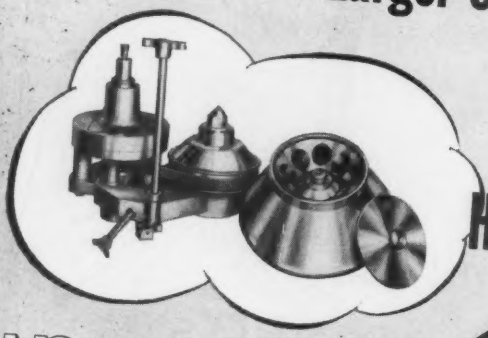

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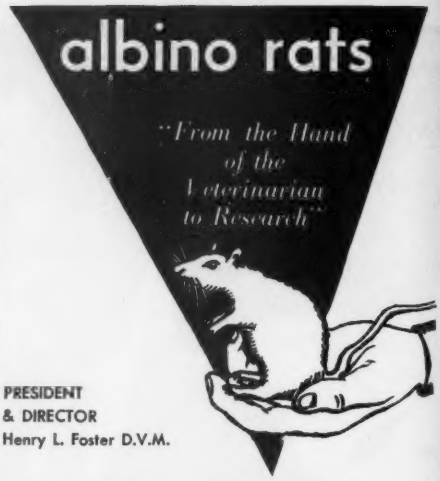

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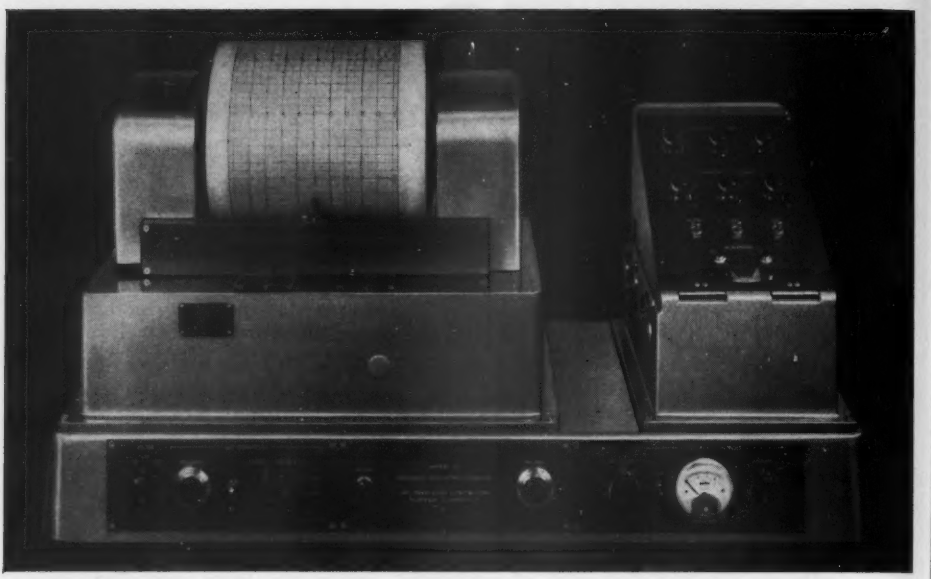

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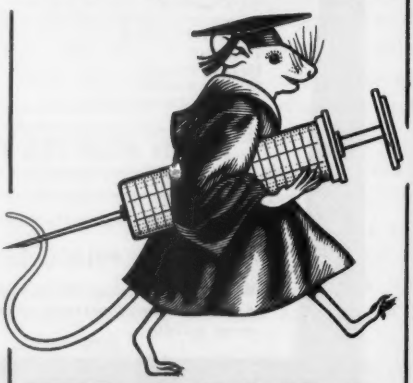

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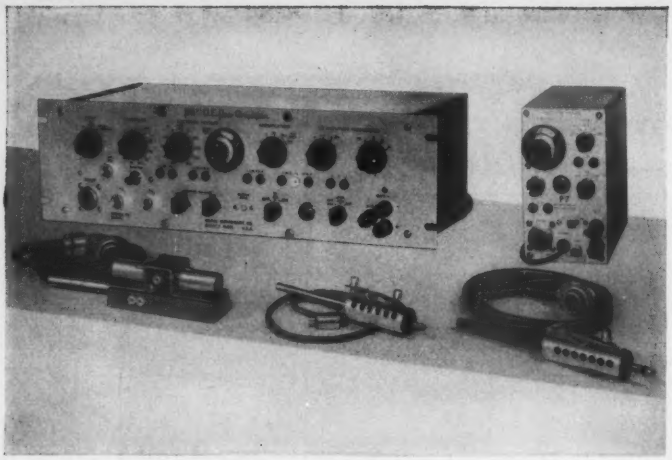

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	5235	500	1-60	2
ulator	8236	20,000	DC-60	20
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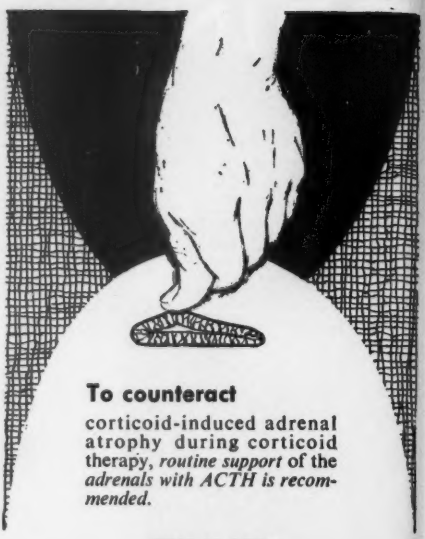

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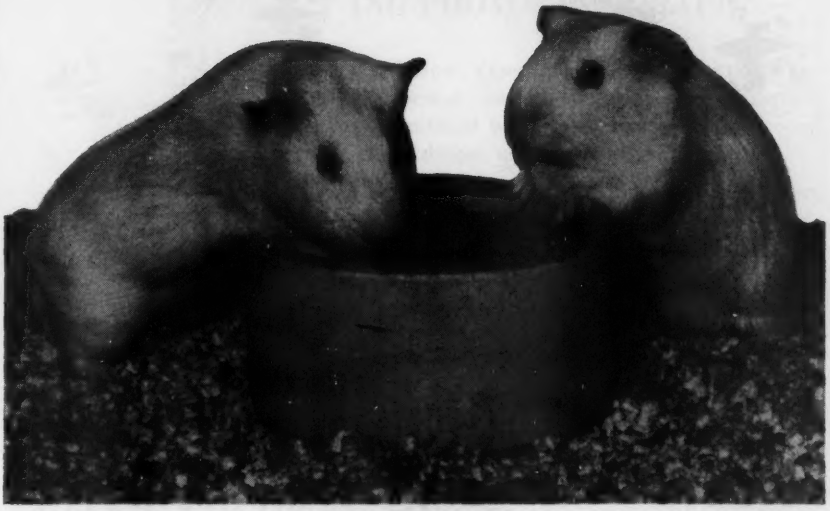

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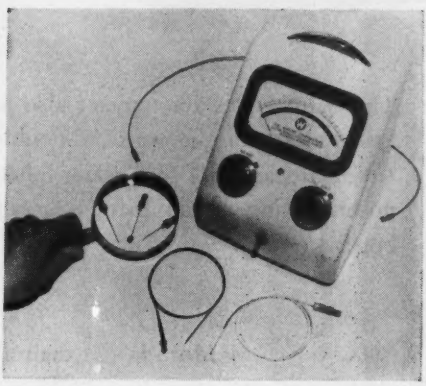

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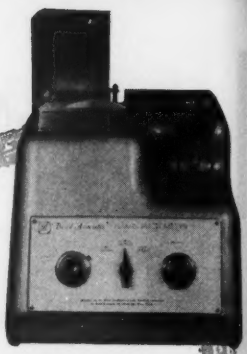

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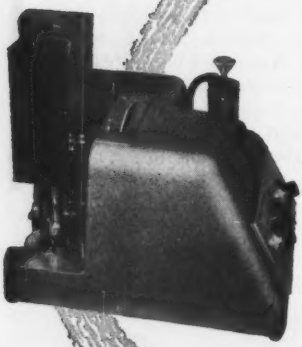

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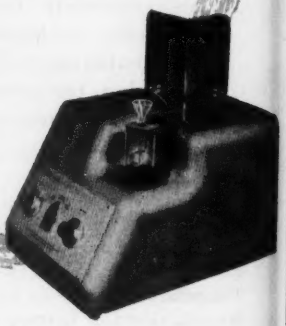

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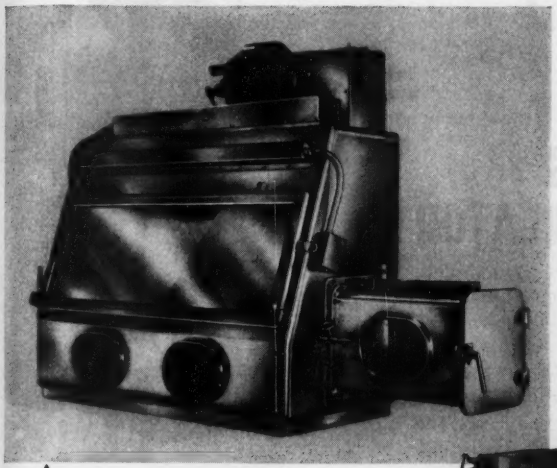

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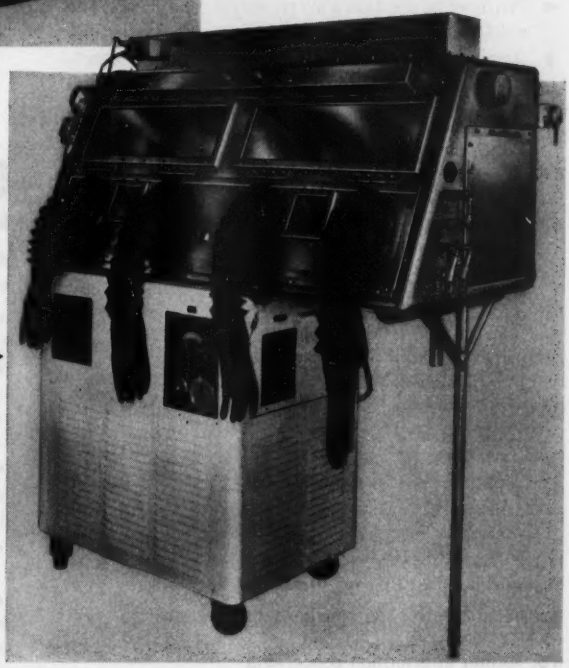

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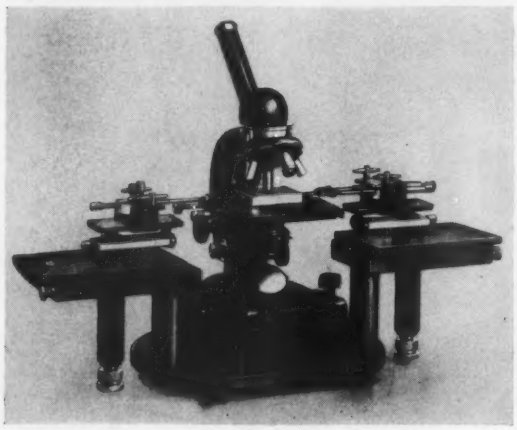

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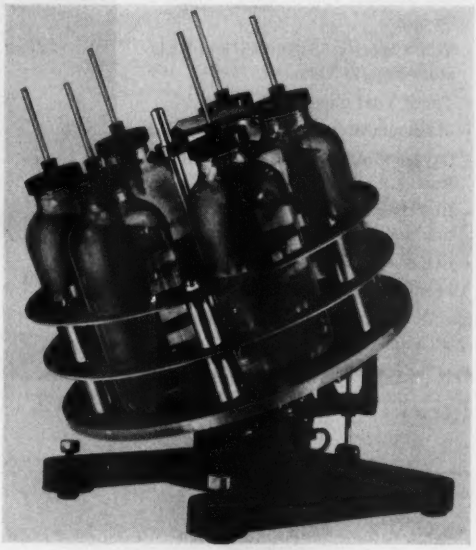

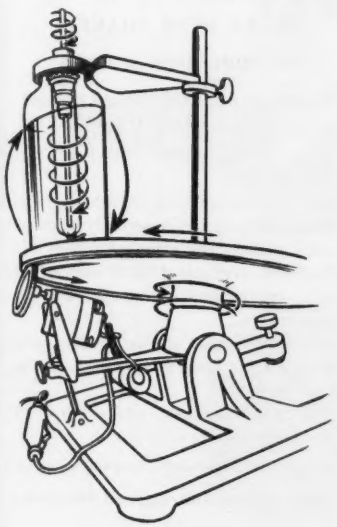

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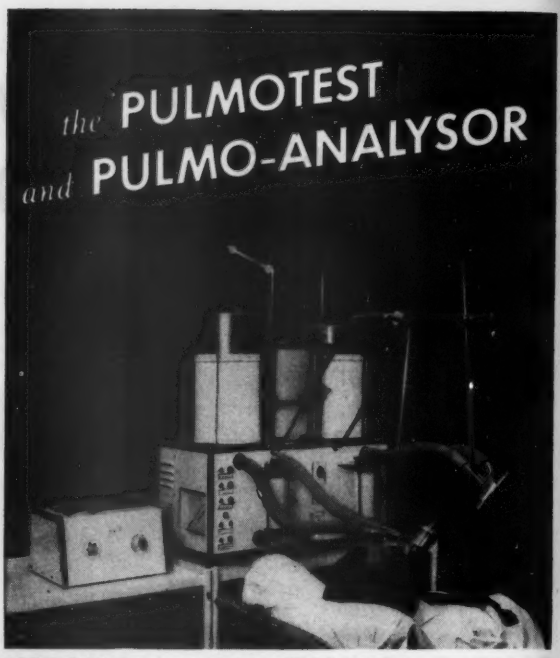

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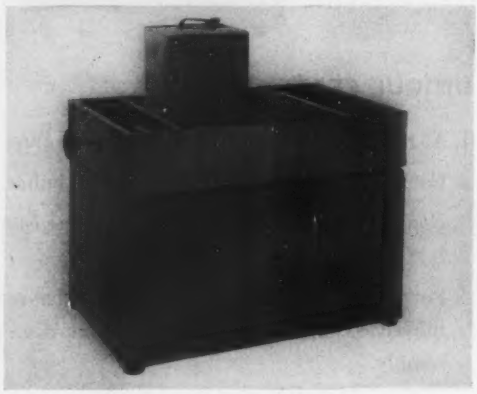

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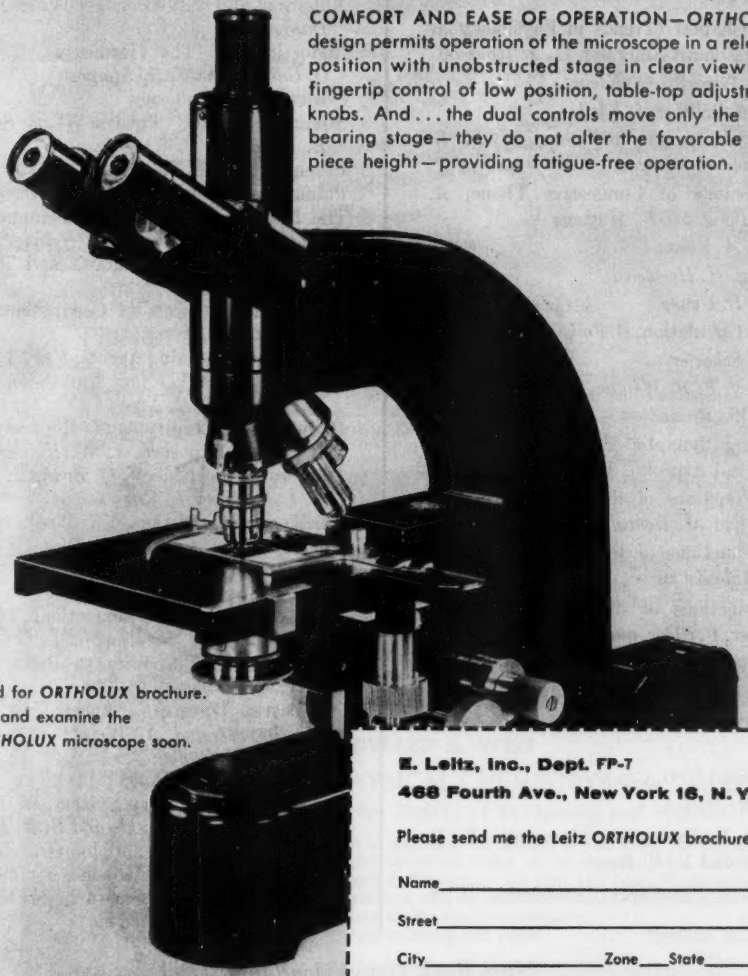

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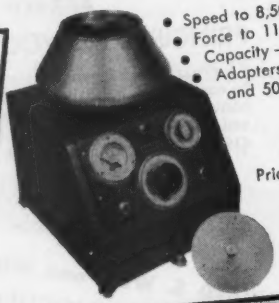

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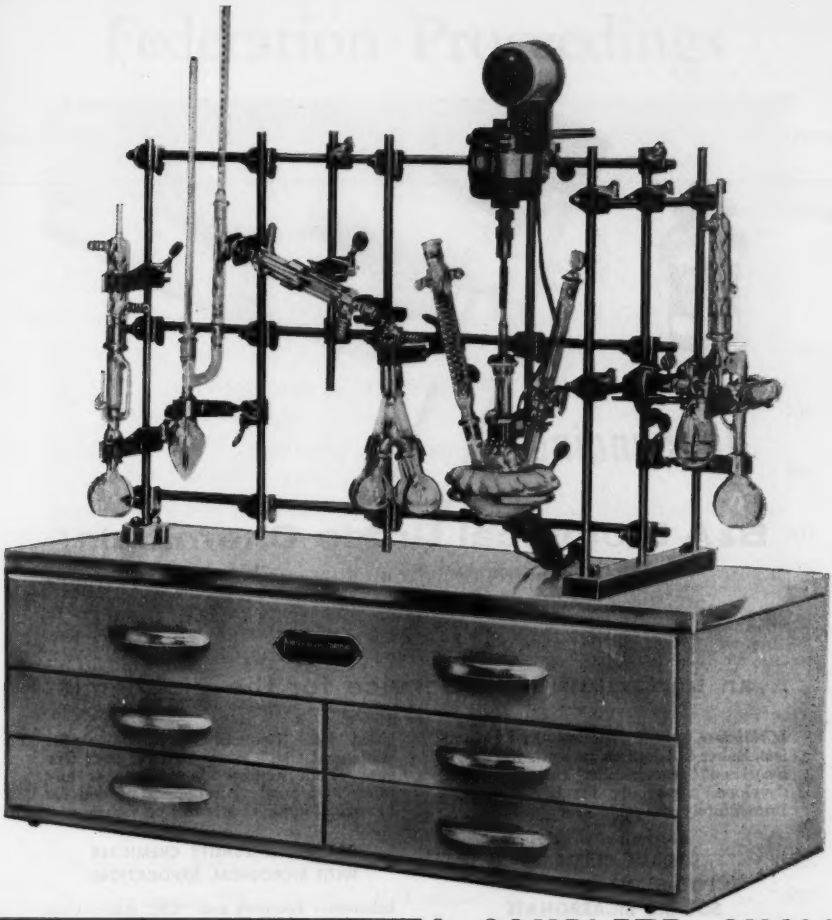

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CONTENTS

AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS: GOLDEN ANNIVERSARY

Introductory Remarks by the President. *J. Murray Luck* 793

Congratulatory Messages on the 50th Anniversary of the American Society of Biological Chemists 795

Origin and Development of the American Society of Biological Chemists. *Philip A. Shaffer* 800

Journal of Biological Chemistry: Recollections of Its Early Years and of Its Founders. *A. N. Richards* 803

Development of International Organization of Biochemistry. *Elmer Stotz* .. 807

Resolution of Past and Present Members of the Editorial Board of the Journal of Biological Chemistry. *Donald D. Van Slyke* 810

SYMPOSIUM ON NUCLEIC ACIDS

Opening Remarks. *Herbert E. Carter* 811

Virus Composition and Structure—25 Years Ago and Now. *Wendell M. Stanley* 812

Progress in Developing Chemical Concepts of Genetic Phenomena. *N. H. Horowitz* 818

Chemical Pathways of Nucleic Acid Biosynthesis. *George Bosworth Brown* . 823

Bacteriophage as Nucleoprotein. *E. A. Evans, Jr.* 827

Enzymatic Synthesis of Ribonucleic Acid-Like Polynucleotides. *Severo Ochoa* 832

Studies on the Structure of Ribonuclease. *Stanford Moore, C. H. W. Hirs and William H. Stein* 840

SYMPOSIUM ON TEACHING PRACTICES IN BIOCHEMISTRY

Introductory Remarks. *J. Murray Luck* 849

Preliminary Survey of the Teaching of Biochemistry in the United States. *Benjamin Harrow* 850

Current Practices in Undergraduate and Graduate Training of Biochemists. *C. A. Elvehjem* 855

Planning a Course in Medical Biochemistry in a New School: Reunion with Physiology. *Abraham White and Henry D. Lauson* 860

The Teaching of Biochemistry in an Integrated Medical Curriculum. *Harland G. Wood* 865

Graduate Training in the Medical Sciences. *Eric G. Ball* 871

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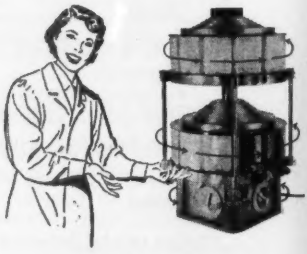

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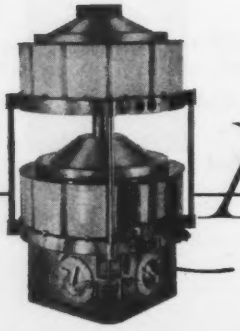

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AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS

Golden Jubilee Celebration[1]

INTRODUCTORY REMARKS

President: J. MURRAY LUCK

LADIES AND GENTLEMEN, FELLOW MEMBERS OF THE AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS, OUR COLLEAGUES FROM OUR SISTER SOCIETIES OF THE FEDERATION, DISTINGUISHED GUESTS:

On behalf of the American Society of Biological Chemists, I welcome you to this the Golden Jubilee Celebration of the Society and of the *Journal of Biological Chemistry*.

This is a year of notable anniversaries: the 25th Anniversary of the Society of Biological Chemists, India; the 30th of the Japanese Biochemical Society; just a few percent short of being the 50th Anniversary of the Biochemical Society and of the *Biochemical Journal*; a very close approximation to the 50th of our own Society and of the *Journal of Biological Chemistry*; the 100th Anniversary of the birth of Russell H. Chittenden, the first president of our Society and incumbent of the first chair of biochemistry in this country; and, finally, according to the *Saturday Review*, the 2500th Anniversary of the birth of Kohmar Pehriad. The first of these anniversaries was celebrated at Agra in January and our Society was fortunate in being represented by our recent past president, Dr. Glen King. The 30th Anniversary of the founding of the Japanese Biochemical Society was appropriately celebrated at Tokyo in November 1955. Dr. Keith Cannan attended as our representative and presented the felicitations of our Society.

Since we intend, with all the immodesty that is appropriate to such an occasion, to bask for the rest of the evening in the glorious achievements of our past 50 years, I intend to say nothing more about the history of the *Journal* and of our Society. Indeed, we have engaged the services of quite a galaxy of the illustrious who are not to feel the least deterred in singing the praises of the Society and of the *Journal*. We hope also to prevail upon Dr. R. E. Davies to say a few words about our sister Society in Great Britain for I think it is noteworthy that the idea of founding a biochemical society should have seized upon the imagination of biochemists on both sides of the Atlantic at about the same time.

I did not know Professor Chittenden—one of that small and noble band through whose efforts the American Society of Biological Chemists came into being. Perhaps he would have looked with askance at the high protein fare that has been ours this evening, but he would have felt gratified to know that the Society had at last shaken off the frivolities of youth and was now settling down to a sedate and comfortable middle age.

I pay tribute to the apocryphal birth of that apocryphal scholar, Kohmar Pehriad, in 544 B.C., only because he is represented to us as the inventor of the comma and the period without which the *Journal of Biological Chemistry* and almost all communicated thought would blush in the nudity of the unpunctuated sentence. Think of the innuendo, the ambiguity, and the wealth of confusion we could so effortlessly put into our writings were it not for that gifted inventor of the small round dot.

Nor do I intend to overlook that doughty present-day champion of the comma and the

[1] Atlantic City, April 18, 1956.

small round dot and his fellow knights with their sharpened lances of graphite—our beloved Editor, Rudolph Anderson, and the members of the Editorial Board. For 25 years he has served on the Editorial Board and since 1937 he has edited the Journal and has communicated his wisdom and superb direction to his fellow members of the Board.

The program does not make mention of a speech by Dr. Donald D. Van Slyke, one of the early members of the Society and a bulwark of defense and support as Managing Editor of the Journal from 1914 to 1925. I intend to call upon him later, perhaps to say a few words about Dr. Anderson. Although this is not the moment to thank the Committee on Arrangements, it is fitting to mention even now, before we begin, that Dr. Van Slyke was the Chairman of the Committee which was responsible for arranging this Anniversary Celebration. On this, he and his colleagues have labored since April last.

I fear that some of you will have accepted my remarks as a Presidential Address, but I trust not. Such trespasses upon the time and patience of the members have been frowned upon for many years, for according to the records the last of these was given in 1915.

Perhaps there are others who, on the contrary, will interpret these remarks to be in fact a Presidential Address. If so, I shall seek comfort in having fulfilled the spirit of an early circular letter to the Council. For in 1912 the Council was reminded that such an innovation [a Presidential Address] "might show to the world at large that the President of our Society is capable of exerting a function."

CONGRATULATORY MESSAGES ON THE 50TH ANNIVERSARY OF THE AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS

Messages from Federated Societies

As President of the American Physiological Society and in *Loco Parentis*, I offer the sincerest and most cordial congratulations to yours, Our Daughter Society, on reaching the fiftieth anniversary of its founding. May past usefulness and harmony foreshadow a bright future and evermore growth in the spirit of Science.

Very sincerely yours,
W. F. HAMILTON, *President*
The American Physiological Society

The membership of the American Society for Pharmacology and Experimental Therapeutics joins me in congratulating the American Society of Biological Chemists on the occasion of its fiftieth anniversary. The constant growth of your Society has contributed in great measure to the solidarity of the Federation of American Societies for Experimental Biology. The kinship of our two societies is well attested by noting that John J. Abel, the second president of your society, became, in the year following his presidency, the founder of our society. The members of the American Society for Pharmacology and Experimental Therapeutics look forward with keen anticipation to many further years of close association with the members of the American Society of Biological Chemists.

E. G. GROSS, *President*
American Society for Pharmacology and Experimental Therapeutics

On behalf of the American Society for Experimental Pathology, it is a privilege to extend our congratulations to the American Society of Biological Chemists on the occasion of the 50th anniversary of the founding of your great chemical society in 1906 under the leadership of its famous chemist, Professor Christian A. Herter. It is particularly fitting that experimental pathologists rejoice in the celebrated accomplishments of your Society, for the first meeting of your Society in 1907 at the George Washington Medical College and the Cosmos Club in Washington, D. C., included dissertations by the experimental pathologists H. Gideon Wells, J. G. Adami, and Oscar Klotz. Philip Shaffer of the Department of Experimental Pathology at Cornell University reported on protein metabolism in exophthalmic goiter. Chemical pathology is an important aspect of the development of experimental pathology that flourishes vividly today in splendid testimony to the wisdom of your leaders and ours.

Sincerely yours,
HAROLD L. STEWART, *President*
The American Society for Experimental Pathology

The American Institute of Nutrition salutes the American Society of Biological Chemists on the occasion of its Fiftieth Anniversary. From its inception the Society assumed a high position of leadership and service. Over the succeeding half century it has grown greatly in numbers, all the while enhancing its prestige. Truly it has achieved an outstanding and enviable record. Its membership has comprised a long line of distinguished scientists whose contributions have had a tremendous import in expanding the total body of useful knowledge. The Institute of Nutrition wishes for the Society of Biological Chemists continued success over the next fifty years.

Yours sincerely,
W. H. SEBRELL, JR., *President*
American Institute of Nutrition

In behalf of the American Association of Immunologists, I wish to offer you our congratulations on the occasion of the Fiftieth Anniversary of the founding of the American Society of Biological Chemists. The magnitude of the contributions of your Society to the progress of biochemistry in this country and all over the world defies description. The successful development of the Federation of American Societies for Experimental Biology is in no small part due to your activities. It is hoped that the progress of your Society will go on at the same rate during the next fifty years as it did during the first half century.

Yours sincerely,
JULES FREUND, *President*
The American Association of Immunologists

Messages from National Biochemical Societies[1]

AUSTRALIA*

The Australian Biochemical Society is probably the youngest biochemical society in the world, and it is therefore understandable that its members should look with admiration at, and gain considerable impetus from, the achievements of the American Society of Biological Chemists over the past fifty years. May I, Sir, on behalf of my Council and the members of the Australian Biochemical Society offer our sincere congratulations to the American Society of Biological Chemists on the occasion of its Golden Anniversary.

R. LEMBERG, *President*

* By P. M. Nossal

BELGIUM*

The Société Belge de Biochemie is happy at being invited to participate in the fiftieth anniversary of the foundation of the American Society of Biological Chemists. As one of the youngest biochemical societies—only five years old—we are pleased to express here our admiration and our heartiest congratulations. Belgian biochemists are happy to have this opportunity of stating publicly their gratitude for all the aid that their American colleagues have always given them and for the warm welcome they have always received in their laboratories. More than a fifth of our members have stayed in American laboratories for various lengths of time. We sincerely hope that the scientific achievements of the American Society of Biological Chemists will continue with the same zest in the future as in the past and express the wish that the ties of friendship that bind American and Belgian biochemists will always grow stronger.

PAUL PUTZEYS, *President*
CLAUDE LIÉBECQ, *Secretary*

* By J. Berthet.

CANADA*

It gives me very great pleasure indeed to be here tonight on this the occasion of the Golden Anniversary Dinner of the Society, to bring greetings and congratulations to you from the Canadian National Committee of Biochemistry. In the moment which I have at my disposal I would like to pay tribute to a great Canadian biochemist, the late Professor A. B. Macallum, who was a member of the original Editorial Board of the *Journal of Biological Chemistry*, and who served for many years in that capacity and also held executive office in the Society for many years, being President in 1912 and 1913.

J. B. COLLIP

CZECHOSLOVAKIA

I take the liberty of transmitting to the members of the Society my message of the most cordial greetings and wishes of full success on the occasion of the Anniversary.

ACADEMICIAN FRANTIŠEK ŠORM
Secretary General

DENMARK*

Dr. Frank Lundquist, of the council of the Danish Biochemical Society, has entrusted me with the honor of conveying the congratulations and best wishes of our Society to the American Society of Biological Chemists on the occasion of its 50th Anniversary. It would be difficult for anyone to compete with the spokesman of our Norwegian brother Society, Ragnar Nicolaysen, in expressing in fitting terms our warm feelings for the inspiration and guidance which the American Society of Biological Chemists has brought us through the years. As you know, Danish biochemists such as Kai Linderstrøm-Lang, Hans Ussing and many others have close ties and mutual friendships with many of the leaders of the American Society of Biological Chemists. Fortunately, perhaps because of these friendships, the traffic has not been just away from Denmark but also toward Denmark, especially to the Carlsberg laboratory, to which we often refer as the 'Parnas.' It is our fondest wish that this traffic in both directions will continue. The greetings and wishes from our Society to yours includes not only professors, doctors and magisters but also pre-magisters. In our charter, at least one of the Council of four is elected from the student group. It is, therefore, an extra pleasure for me to serve as the direct spokesman for the youngest generation of Danish biochemists on this occasion. All of us wish to congratulate you on the achievements of the past 50 years and look forward to the continuing growth of American biochemistry in the next 50 years.

* By H. M. KALCKAR.

[1] Those marked with an asterisk were read at the 50th Anniversary Dinner.

FINLAND*

For its fifty years of successful activity the American Society of Biological Chemists is cordially greeted and congratulated by biochemists and microbiologists of Finland. The *Journal of Biological Chemistry*, the important publication of your Society, has throughout its existence been of extreme benefit to biochemical research all over the world.

A. I. VIRTANEN, *President*

FRANCE*

La Société de Chimie Biologique apprécie profondément l'honneur qui lui a été fait de participer aux manifestations du Jubilé de l'American Society of Biological Chemists. Aussi nous nous réjouissons de pouvoir associer les Biochimistes français aux témoignages d'amitié et de sympathie, qui vous sont adressés de toutes les parties du monde. L'American Society of Biological Chemists a été créee au moment où la Biochimie commençait d'être une Science Autonome. La Société Américaine a joué un rôle de premier plan dans l'evolution de la Biochimie, aussi bien par le nombre de ses adhérents, que par leur valeur. Il nous parait particulièrement heureux d'avoir commémoré en même temps le cinquantenaire de la plus importante Société Biochimique du monde et du plus prestigieux périodique relevant de cette science. En France, nous avons la plus grande estime pour le *Journal of Biological Chemistry*, qui a si largement contribué aux progrès de la Biochimie. Nous le lisons avec un très vif intéret, car si depuis 1906 le nombre des articles publics chaque année s'est notablement accru, leur qualité s'est constamment maintenue au même niveau: le plus elevé. Depuis sa création en 1914, la Société de Chimie Biologique entretient d'amicales relations avec l'American Society of Biological Chemists. Les récents Congrès Internationaux de Biochemie ont permis de multiples contacts des plus cordiaux entre membres de nos deux Sociétés. C'est au nom de cette solide et durable amitié, que nous nous associons de grand coeur aux adresses de felicitations que vous recevrez, nous désirons vous exprimer nos sentiments d'estime pour l'œuvre que vous avez accomplie. Nous y joignons tous les vœux chalcureux, que nous formons pour que l'avenir de l'American Society of Biological Chemists et du *Journal of Biological Chemistry* s'affirme toujours de plus en plus prospère.

P. BOULANGER, *Le Président*
J. E. COURTOIS, *Le Sécretaire Général*
Y. RAOUL, *Le Rédacteur en Chef, du Bulletin de la Société de Chimie Biologique*

* By J. E. Courtois.

GERMANY*

The Society for Physiological Chemistry in Germany wishes to compliment the American Society of Biological Chemists on its 50th Anniversary. During these 50 years Biochemistry has become one of the outstanding fields of natural science. When your society was founded the formulas for saccharides, nucleic bases and most amino acids had already been established and in intermediate metabolism the first approaches towards the elucidation of the decomposition of fatty and amino acids were made. Today we have extensive knowledge of the structure of a protein molecule, the sequence of amino acids, the composition and biological function of nucleic acids and above all, our conception of intermediate metabolism and the involved enzymes and coenzymes has become extremely broad and profound. The American Society of Biological Chemists can be proud that its members have contributed so much to the development of our branch of science. Our wish is that the achievements of your society in the future may even exceed those of the past.

KURT FELIX, *President*
N. VOIGT, *Secretary*

* By L. Roka.

GREAT BRITAIN*

This Society desires to convey its best wishes to the American Society of Biological Chemists and its most sincere congratulations on the achievement of the Fiftieth Anniversary of the *Journal of Biological Chemistry*. On this memorable occasion it is pleasant to recall that a link existed between our two Societies from their very beginnings in the dawn of modern biochemistry. It is recorded in our history that the purchase of the *Biochemical Journal* from its private owners by the Biochemical Club, which thereupon became the Biochemical Society, was helped by a generous gift from Mrs. Christian Herter. Henry Drysdale Dakin, through whose good offices this gift was received, was an Original Member of our Society and for more than forty years formed a living link between us. He was but the first of many famous men who later honoured both Societies with their membership. We are therefore entitled today to share your proper pride in past achievements and join in your hopes for an even more brilliant future.

F. L. WARREN, C. E. DALGLIESH
Honorary Secretaries

* By R. E. Davies.

INDIA*

In behalf of the Society of Biological Chemists, India, I would extend to you our warmest felicitations on the happy occasion of the Anniversary of the founding of the American Society of Biological Chemists. The period of the last fifty years has witnessed phenomenal advances in Biochemistry and to the attainment of this position the work of American scientists has made outstanding contribution. We have every confidence that their future contributions will be of equal and even greater importance. Let us on this occasion forge yet stronger bonds of fellowship among the biochemists of the world.

B. C. GUHA, *President*
Society of Biological Chemists, India

* By P. M. Bhargava.

ITALY

I have the great pleasure, on behalf of the Italian Society of Biochemistry, to send the most sincere greetings to your Society, on the occasion of the Fiftieth Anniversary of its foundation. We all know and appreciate the most important, essential contributions derived from your Association's members to the progress of our science, and the best wishes we may think of on this glorious occasion are to see continued in the years to come the leading function which American biochemistry, through your Society's members, has acquired today.

G. QUAGLIARIELLO, *President*

JAPAN*

On this memorable occasion of the 50th Anniversaries of the American Society of Biological Chemists and the *Journal of Biological Chemistry* I have the honor of representing your colleagues in the Japanese Biochemical Society who send you their warmest congratulations. As Dr. Luck mentioned, last year for the 30th Anniversary of our society we had the great pleasure of receiving the official representative of your Society, Dr. Keith Cannon. Therefore I have the added gratification of being able to reciprocate your cordial gesture. Through the *Journal of Biological Chemistry*, we have followed the outstanding contributions of American biochemists that have led to your position of world leadership in our science. We biochemists of Japan have been much influenced and helped by exchange professorships and fellowships between our countries and the fact that many Japanese biochemists have received training in the United States. I wish to take this opportunity to express on behalf of the Japanese biochemists, our profound appreciation. We wish you in the next fifty years as much progress and success as you have experienced in the past half century.

* By Norio Shimazono, for the Japanese Biochemical Society.

NETHERLANDS

The Dutch Biochemical Society offers her best wishes on the occasion of your Fiftieth Anniversary. May you continue adding the wisdom of fifty years experience to the wisdom of hope you always possessed.

LENS, *President*

NORWAY*

It is a privilege to have been asked to address this illustrious society on such an occasion, although with much regret it has to be done across the four thousand miles of Atlantic Ocean. However, one real privilege of scientists is that no oceans, no walls, separate us in reality. We all know each other and we are old friends whenever we meet. As a matter of fact no one could have made this clearer to me than one of your renowned members: Baird Hastings. When we met first about 13 years ago he patted my shoulder and said, "By Jove, we are old friends; I have known you for ten years." Your periodical, the *Journal of Biological Chemistry*, is like a continuous stream of greetings to us in our part of the world, in fact to all admirers of American biochemistry everywhere. Our job is to find facts by intelligent speculation followed by experimentation, and nothing but the truth will satisfy us. Time has marched since the birth of your society and truly you have grown at an exponential rate in every way, to the benefit and to the inspiration of us all. The warmest greetings and congratulations from all Norwegian biochemists accompany these words from your old friend.

RAGNAR NICOLAYSEN, *Chairman*
Norwegian Committee on Biochemistry

POLAND

The Fiftieth Anniversary of your Society represents a milestone in the history of Biochemistry. In assessing the achievements of modern biochemistry you may well be proud of the magnificent contributions conveyed to the reader in your *Journal of Biological Chemistry* during the past 50 years. The present generation of Polish biochemists is indebted to a considerable extent to your publication. We extend to you our warmest greetings on the occasion of the Golden Anniversary of your Society and your Journal. We are convinced that the extension of contact between biochemists

of our two countries as well as all other countries should, and will, continue to increase and that such cooperation is in the best interest of science and mankind.

For the Biochemical Committee
JOZEF HELLER, *Chairman*
JERZY MEDUSKI, *Secretary*

SPAIN*

The Spanish National Committee of Biochemistry wishes to congratulate the American Society of Biological Chemists in its Fiftieth Anniversary. Your tremendous success in this period of time is for our young Spanish Society a shining example and a matter of hope. On our own scale, we would be very happy to follow your lines. After the second World War a number of Spanish students have come to the United States for training in biochemical research. We would like to see this number increase. This would be of primary importance for the development of biochemistry in Spain. We would be very grateful for your cooperation in making it possible for our students in biochemistry to complete their training with you. Finally we wish to invite you to Spain. As you know we have in Spain a great deal of history, folklore and scenery. Now we also have the beginning of a generation of biochemists. Even purely tourist visits would be useful for us, since they would offer opportunities for example, advice and increase in mutual understanding. You will be very welcome!

A. SANTOS-RUIZ, *President*

* By A. Sols.

SWEDEN

On the occasion of the Fiftieth Anniversary of the American Society of Biological Chemists the Swedish National Committee for Biochemistry sends cordial congratulations and best wishes for the future.

HUGO THEORELL, *Chairman*
KARL MYRBÄCK, *Secretary*

ORIGIN AND DEVELOPMENT OF THE AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS

PHILIP A. SHAFFER

From the Department of Biological Chemistry, Washington University, St. Louis, Missouri

I CAN THINK of no greater honor for an elder member of this group than this privilege of addressing members of all societies of our Federation as a representative of one of them—the Society of Biological Chemists—on the occasion of its 50th Anniversary.

Once a member of three of these societies and an occasional dabbler in fields represented by others, I am aware of our common interests and of the value of the Federation which unites us all in cooperation; this is an opportunity to acknowledge, as societies and as individuals, our indebtedness to the Federation—and to the officers of our societies, including the hard-working secretaries.

To present a 'Résumé of the Origin and Development of the Society of Biological Chemists,' as I am asked to do, requires an interpretation broad enough to recognize the relationship of our Society to its direct ancestors: the American Chemical Society and, particularly, the American Physiological Society.

When the Physiological Society was created in 1887 by H. P. Bowditch, Weir Mitchell and H. N. Martin, they invited two physiological chemists to be among the 28 founding members—Chittenden and Vaughan. This recognition of the close relation between physiology and physiological chemistry was a boon to the new Society and also stimulated the growing interest in biochemistry. Nineteen years later when John J. Abel proposed creation of a society of biological chemists, at least 52 of those who became its charter members were already members of the Physiological Society.

At its celebration meeting in 1938 at Baltimore, Professor Chittenden was called upon and his response deserves quoting on this occasion: "It is one of the glories of the American Physiological Society that at its very inception it took into its fold physiological chemistry and that at a time when that discipline, like a stray child, was wandering about not knowing where to go or where it belonged. Today physiological chemistry—or perhaps you prefer to call it biochemistry—is reaching out in all directions, throwing light on many of the mysteries of life and giving health and strength in many ways to its foster mother Physiology."

In 1906, John J. Abel of Johns Hopkins was insisting that biochemists were then numerous enough and strong enough to leave the shelter of parent societies and have an association of their own. Chittenden agreed with him. They conferred and, on December 13, Dr. Abel sent letters to those they thought would be interested, inviting them to attend a meeting on December 28 in the quarters of the American Association for the Advancement of Sciences in the Hotel Belmont, New York City, to consider organizing a society of biological chemists.

Twenty-nine attended. They are the 'truly' charter members. Four of them are now living. They are: *Samuel Amberg*, Mayo Clinic; *William J. Gies* who acted as secretary at that first meeting and was forthwith elected to that office in the society; *Alfred N. Richards*, our elder statesman, the second Secretary of this Society and responsible, more than anyone else, for the continued success and idealism of our Journal; and *Philip A. Shaffer*, odd jobs man through the years.

At that first meeting Dr. Abel made an enthusiastically accepted address, presenting arguments which convinced those present of the need and purpose for creating a new, independent society. 'Articles of Agreement' which he had prepared were unanimously adopted and organization was effected. That was the 'origin' per se. The officers and council members elected were: R. H. Chittenden, *President*; John J. Abel,

Vice-President; W. J. Gies, *Secretary*; L. B. Mendel, *Treasurer*; Otto Folin, W. Jones, W. Koch, *Councilors*.

That meeting is covered in a report written by our first secretary, Dr. Gies, and published in *Science*, volume 25, January 1907. I have here a tattered reprint of that article, also the sheet of Articles of Agreement. At the top of that sheet, in large letters, is the name of the society—characteristically, The American Society of Biological Chemists—*not* biological chemistry but biological chemists—individuals working in a common but varied field unhampered by bonds of traditional attitudes.

Others who indicated their wish to join but who could not be present were rated as charter members also and brought the total up to 81. I would not cite the present large membership in comparison with that of 50 years ago as a measure of our growth but rather the notable achievements of our members.

What happened next after that first meeting and the record of achievement through the early years is covered in Chittenden's volume, *First Twenty-Five Years*. Since this is a social and not a scientific session, it would be inappropriate as well as impossible in time to duplicate here that detail of biography and achievements, but progress during that period may be illustrated by a few topics of large significance in that they revealed *promise* of solution.

1) Proof of the existence of Vitamins, Enzymes and Hormones. Many of each class were isolated and their activity demonstrated but their chemical *structure* was in only a few cases discovered. The *manner* of their action was only surmised.

2) Another important field of accomplishment was the invention of simple and more precise methods of quantitative analysis, applicable to small volumes of tissue or body fluids, needed to reveal changes of concentration of metabolic constituents in experimental animals—or persons. Such new semi-micro methods as devised by Otto Folin, D. D. Van Slyke, Stanley Benedict and many others at once became useful aids to clinical diagnosis. That usefulness to many fields of medicine, as well as to experimental studies, was a large influence in the increasing prestige of bio-chemistry in almost every medical school in the country. The boom for bio-chemistry was under way!

One illustration from my personal experience: when in 1910 I went to the Department of Biochemistry in Washington University in St. Louis, research in the subject was limited to that Department; almost, one might say, tolerated in that Department. Now there is not one department in the whole medical center that does not rely heavily upon bio-chemistry; many of them now are carrying on major projects in biochemistry as the realistic approach to the solution of their problems.

In the second 25 years, only just now being completed, we are able to record illustrations of achievement hoped for but hardly dreamed possible 25 years ago. For instance, illustrations taken from the program of this meeting in 1956; at the Anniversary Symposium on Tuesday, many of you heard the following topics presented: 'The Composition and Structure of Virus,' 'Chemical Concepts of Genetic Phenomena,' 'The Pathway of Nucleic Acids Bio Synthesis,' 'Bacteriophage as Nucleo-Proteins,' 'Enzymatic Syntheses' and 'The Structure of Enzymes.'

What greater proof is needed of the widening scope and stimulating progress within the Society's membership? Each would acknowledge that the work of many others in earlier years produced the foundation on which their research could develop and progress more significantly.

The following volumes record that evolution of our own and sister societies:

A Half Century of Chemistry in America, published in 1926, vol. 48, of the Journal of the American Chemical Society. The chapter written by Graham Lusk, 'The Chemistry of Physiology and Nutrition', describes the transition that occurred around 1900 when physiological chemistry based upon experimentation began to replace the traditions and complacency of the 'old-fashioned' medical chemistry.

In 1930 *Development of Physiological Chemistry in the United States*, by Chittenden was published. This was supplemented by his *First Twenty-Five Years of the American Society of Biological Chemists*.

The History of the American Physiological Society, by W. H. Howell and Charles W. Greene, was published in 1938 following the semi-centennial celebration in Baltimore. (I have here a souvenir presented to those present at that meeting—first of our Federation members to celebrate a Golden Anniversary; this is the second.)

Personally I became interested in these early stirrings through my grandfather for whom I was named and whose scientific interests are

perhaps responsible for my own. He was graduated a year after Weir Mitchell from Jefferson Medical College where he studied under Weir Mitchell's father, who signed his diploma. You know the part Weir Mitchell had in founding the American Physiological Society and of the injustices he had suffered in consequence of his preaching what he practiced—"experiment, investigate, find out for yourself what you do not know."

That has been the custom bio-chemistry and physiology have thrived on ever since 1871 when the physiologist Bowditch, of Harvard, with the help of chemist-president Eliot, secured the first laboratory and equipment to be devoted to experimental work, followed soon by a similar establishment at Johns Hopkins by Newell Martin, and by two others devoted primarily to technical aspects of physiological phenomena and called physiological chemistry; these were in Michigan and Yale by Vaughan and Chittenden, respectively. Their influence began to spread as assistants went to other institutions—Chandler and Gies to Columbia, Hawk to Marshall's laboratory in Philadelphia, etc. It all had to wait until laboratories (now taken for granted) became available to permit experimental methods essential for all substantial progress in physical sciences.

In 1900 I became Otto Folin's first assistant in a chemical laboratory for research at McLean Hospital near Boston. It was one of the first in this country to be established in hospitals for research in pathological chemistry.

With the resulting upsurge in this field all over the country, significant names became too numerous to mention here. But I have brought with me an interesting exhibit—two group photographs—one, taken of all who attended the annual meeting of the Federation held in St. Louis in 1914, one year after the Federation was created in Philadelphia and when our Society was only eight years old; the other in 1926 when the Federation again met in St. Louis. I know of no other such pictures of our Federation. I think you will enjoy getting acquainted in this way with members you may have known only by name, if you are young, or in renewing old acquaintances if you are older.

Many of the faces have been identified by contemporaries and listed. I solicit your attention to the pictures and your contribution to the list of identifications.

By way of honoring all of our members who have received notable recognition let us pay tribute to the most recent recipient of laurels—winner of the Nobel Prize—whose recent achievements seem to promise important progress in the near future—his synthesis of 'Oxytocin', a hormone of the pituitary gland—the first synthesis of a polypeptide hormone. This was accomplished in 1954 by Vincent Du Vigneaud with the aid of four assistants, two of whom are scholars on leave from Australia and Greece, a clear indication of the role of this country in biochemistry today. For this Du Vigneaud was awarded the Nobel Prize. In saluting him we pay tribute to all those who have gone before.

JOURNAL OF BIOLOGICAL CHEMISTRY: RECOLLECTIONS OF ITS EARLY YEARS AND OF ITS FOUNDERS

A. N. Richards

From the University of Pennsylvania Medical School, Philadelphia, Pennsylvania

Fifty-three years ago, an exchange of letters took place between John J. Abel of Baltimore and Christian A. Herter of New York; they contain the germ of the *Journal of Biological Chemistry*. Abel's letter cannot be found; hence I quote only from Herter's reply, dated May 24, 1903—

"I am a good deal interested in your letter suggesting the propriety of a new medium for the publication of biochemical writings in this country. A similar idea has often been in my mind although I never had any clear view as to the best way of reaching the desired end. It is inevitable that much good material for a journal devoted to chemical problems in physiology and pathology should be forthcoming in this country and I cannot see where it is to be printed unless there is some new publication for the purpose. . . ."

"To restate my position then, I would say that I favor the establishment of a journal such as you have in mind and will gladly help along in any way I can."

There are no records of progress during the ensuing year. Herter spent a large part of it in Europe. But it is certain that during that time many colleagues, here and abroad, were consulted and gave encouragement. Upon Herter's return discussions were resumed and it was agreed to go ahead. Scientific backing was secured from more than 20 active investigators in almost as many institutions; they became collaborating editors. Responsible editorship was jointly assumed by Abel and Herter. I was Herter's assistant at Columbia at that time and it was convenient to give me the tasks of assistant editor.

To guarantee permanence, the Journal was incorporated as a non-profit stock company, the members of which were Herter, Abel, Edward K. Dunham, Reid Hunt and A. N. Richards. Each held one share of stock, endorsed with the agreement that it could not be transferred except with the permission of three other members. The members elected themselves directors; the directors chose Herter to be president; Dunham, treasurer; Richards, secretary. The corporation continued to own the Journal until it was transferred to the Society in 1942.

It was decided that the format of the Journal should closely resemble that of Hoppe-Seyler's *Zeitschrift für physiologische Chemie* and that the price to subscribers should be so low that young workers could afford it.

Financial deficits were foreseen as inevitable. Various means of meeting them were explored and discarded. One of these is worth mention for it illustrates policy. It appears that a proposal came to Abel from the Secretary of the American Medical Association, indicating the willingness of that organization to assume the financial burden with the implication, I believe, of participating in ownership. It was not accepted because of the determination that the new Journal should never enter into alliances outside the field of biochemistry but should always be managed by and for biochemists, solely for the advancement of that science. In the end it was left to Dr. Herter to make up the deficits, and this he did until his death five years later.

In the summer of 1905 hundreds of announcements of the projected journal were printed and mailed to libraries, laboratories and individuals known to have a working interest in the science. The mailing was done from Dr. Herter's summer home in Maine, the whole family taking part in that job, including his young daughters and his nephew and namesake, now the Governor of Massachusetts.

October of 1905 was the time scheduled for the first number. Five papers, enough for one number had accumulated; a list of 130 subscribers had been obtained. Osler was the first subscriber from Great Britain; Calmette, the first from France. Inexperience of printers and of the assistant editor caused exasperating delays. Herter wrote to Abel, "It's simply wonderful how many there are." But finally, on or about October 20, it did come out and everyone was relieved and proud. The first article was by Abel and Taveau on epinephrine; the second was by Reid Hunt on the action of thyroid on the

toxicity of acetonitrile. Its opening paragraph gives an idea of how remote 1905 actually is:

> "Speculation as to the function of the thyroid glands had taken two main directions; some suppose that these organs elaborate an internal secretion which is of itself necessary for certain processes of metabolism, while others think that their chief function is to neutralize poisonous substances originating in the body ('enterotoxines') or introduced into it from without (iodine, for example)."

The three following issues brought Volume 1 to completion in June 1906. In it is to be found the first paper by Dakin published in this country; one by Treat Johnson and young E. V. McCollum on pyrimidines; four short papers by Herter on chemical products of intestinal bacteria; and one by Jacques Loeb on the antagonism of Mg and Ca ions.

The second volume, completed in January 1907, went to 300 subscribers, more than double the first list. After that volume, Waverly Press became our printers as they still are. The *JBC* was their first scientific journal.

Upon the completion of Volume 6, in February 1909, Dr. Abel resigned his editorship to devote his energy to the formation of the American Society of Pharmacology and Therapeutics and to the initiation of its journal. He continued as a collaborating editor. His resignation left Dr. Herter as sole editor until his death in 1910.

Herter's death, at the age of 45, so untimely, was a tragic loss to the Journal and necessitated important changes. Simon Flexner was made president of the Journal corporation; the editorship was taken over by a board, elected by the Directors—H. D. Dakin, E. K. Dunham, Lafayette Mendel and A. N. Richards. The truly editorial duties were distributed among the four; the business management was transferred to rooms in my laboratory, loaned by the Trustees of the University of Pennsylvania. I was given the luxury of a secretary and obtained editorial assistance from the person who later attained distinction as librarian of the Marine Biological Laboratory at Woods Hole, Mrs. Thomas H. Montgomery.

Dr. Herter's death entailed not only the loss of a much-loved and highly gifted leader but also the cessation of his financial support. The situation was critical and means had quickly to be found either to enlist new support or to devise ways of making the Journal self-supporting. Both were realized.

Thanks to the editors' belief in its future, the printed editions of the early volumes far exceeded the number of subscribers. An offer was made to scores, perhaps hundreds, of libraries here and abroad to give to any one of them a set of the first ten volumes at no cost, provided they would agree to subscribe to subsequent volumes at published price. A little later a similar offer was made to supply the first thirteen volumes at nominal cost to any individual biochemist who would subscribe for subsequent issues. These devices, together with economies in management, brought the budget into balance.

During that same period relatives and friends of Dr. and Mrs. Herter assembled a fund of $40,000 as a tribute to Dr. Herter's memory. It was not necessary to deplete that fund and so it still remains, with accrued increases, as a continuing backlog of financial strength. Neither was it necessary to accept either of two offers of help which came, one from the American Medical Association and one from the American Chemical Society. Acceptance of either would have jeopardized the policy of independence adopted at the outset.

The management of the Journal remained at the University of Pennsylvania for four years. Two volumes came to be the yearly output. That doesn't seem large compared with the present rate of six great volumes a year. But it was more than I could manage and at the same time meet my obligations to the University. So, to the great good fortune of all concerned, the duties of managing editor were accepted by Donald Van Slyke of the Rockefeller Hospital. The event shows that the Journal had become a going concern.

I feel intense and lasting pride that it was my unique privilege to help into print the work of many great American investigators, some in the early period of their careers. The names of some who are no longer with us whose work will long endure stand out in my memory—Abel, Hunt, Dakin, Levene, Folin, Treat Johnson, Walter Jones, Stanley Benedict, L. J. Henderson, Walter Palmer, T. B. Osborne and Lafayette Mendel.

I propose to devote the rest of my time to an account, necessarily brief and certainly far from adequate, of the careers and qualities of the two men who started the Journal and with whom I was privileged to work. Abel, the elder, lived to the age of 81; he died 18 years ago. Herter, 9 years younger, died in his 45th year, more than 45 years ago. Certainly very few in this audience could have known Herter; many doubtless did

not know Abel. I feel an obligation to attempt to renew their memory.

The two men were alike in their love of science, in their faith in its future as the basis of the advance of medicine and in their obedience to the sense of duty which that faith imposed. In other respects they were as widely different, it seems to me, as two human beings could be—upbringing, cultural background, education and professional careers. Yet they were close friends and congenial collaborators.

Abel, born in Cleveland and brought up austerely, was obliged to make his own way through the college of the University of Michigan; he interrupted his course for three years to earn money by teaching in public schools. His interest in chemistry was excited by Victor Vaughan; in physiology, by Henry Sewell—both great teachers. Doubtless it was their influence which sent him, for the year after his graduation, to Johns Hopkins to study under Newell Martin, a brilliant physiologist from the Cambridge school. He decided to study medicine in Europe, where the sciences of medicine were immeasurably further advanced than here and spent the next seven years of his training in seven different European universities. Among his teachers were giants of that era: Ludwig, His, Strümpell, Kussmaul, v. Recklinghausen, Naunyn, Hoppe-Seyler, Nothnagel, Nencki, Schmiedeberg and Drechsel. He received his medical degree from the University of Strassburg, his dissertation having been written under the guidance of Carl Ludwig at Leipzig. He came home in 1890 expecting to practice medicine, but was diverted by a call from President Angell of Michigan to inaugurate a department of pharmacology in that university; it was the first in this country. Two years later, (1893) he accepted Osler's invitation to undertake a similar task at the Johns Hopkins Medical School. His subsequent career of 45 years was spent there.

Herter's early history presents a striking contrast. His father was a gifted, successful architect in New York whose career was cut short by death at the age of 44. His home was a center of artistic rather than scientific culture. The son, Christian, got the equivalent of college training from private instruction under his father's guidance. From early boyhood, medicine was the goal of his ambition. He entered the College of Physicians and Surgeons at Columbia University in 1881 at the age of 15; completed the course with distinction in 3 years; then served an internship at the Bellevue Hospital.

The faculty of that day at P & S was outstanding in this country, though scarcely to be compared with those of the great universities of Europe. Still remembered are the names of Chandler in chemistry, Dalton in physiology, Delafield in pathology and medicine, Jacobi in diseases of children. T. Mitchell Prudden was director of the pathological laboratory; Herter's preceptor was William S. Halsted, then a demonstrator of anatomy.

After his internship Herter studied pathology for a year in Baltimore under William H. Welch; then, a year in nervous and mental diseases at Zurich under Forel, the leading European authority.

On his return to America (1887 or -8) he began the practice of medicine. For a few years his interest centered on patients with nervous and mental diseases and, with Pierce Clark, he published a book on the diagnosis of those diseases. It is said that later he found the study of the nervous system so abounding in confusions that he turned his attention to chemical problems, particularly those connected with pathological conditions. He equipped a laboratory in his own house, primarily for the study of his patients. As his interest in fundamental problems increased, he engaged a succession of well-trained chemists to work in his laboratory and with their help trained himself in chemistry. It was there that all of his chemical and bacteriological work was done. The greater part of Dakin's career was achieved in that laboratory.

Herter's developed competence in pathological chemistry obtained for him the professorship in that subject at the University and Bellevue Medical College in 1898. His published *Lectures on Chemical Pathology* was a book unique for its time. In 1903 he was appointed Professor of Pharmacology and Therapeutics at Columbia. After a year in Europe with Paul Ehrlich and Hans Meyer, he assumed the duties of that position and discharged them for the six years before his death.

In preceding paragraphs, I have pictured Abel as a man, rigorously trained in physiology, chemistry and the chemical aspects of pharmacology who, mature at the age of 34, began a career of vigorous, incessant investigation and teaching of pharmacology which was to continue, uninterrupted, for 47 years.

In contrast, Herter, with little basic training in the sciences of medicine, undertook to teach himself chemistry, first to aid him in problems of practice, then to extend his knowledge of pa-

thology. Finally he was led into the field of pharmacology and developed it for student audiences as a basis of rational therapy and a mode of thinking of medical problems.

Many of Abel's researches may truly be called pioneering. I mention adrenaline, amanita poisons, poisons extractable from toad's skin, phthaleins, dialysis of circulating blood (vivi-diffusion), pituitary hormones, chemistry of insulin, tetanus toxin. In these he was associated with a succession of gifted younger men, who went to him for inspiration and guidance; many of them went on to distinguished careers—to mention a few, Loevenhart, Ford, Rountree, Barbour, Geiling, Chen, Macht, Lamson, Marshall, du Vigneaud. As a teacher, he was particularly effective in the laboratory, in informal discussions and through his example. He advised his students to come to his lectures only if they thought them useful; but he insisted on attendance upon laboratory exercises and demonstrations. No one in this country has had greater influence in bringing pharmacology to maturity as a science.

He had a talent for organization—witness the two departments which he created, numbers one and two in this country; the *Journal of Experimental Medicine* was inaugurated in accordance with a proposal and a plan formulated by Abel; he was the actual founder of the American Society of Biological Chemists and the Society of Pharmacology and Experimental Therapeutics; he played a leading part in the creation of the journals of those two sciences.

Herter's researches were far less consecutive, designed not so much to advance a recognized medical discipline as to help his own understanding of medical problems which excited his especial interest. Some of his chemical studies now seem to chemists inconsequential but to the medical community they were the expression of the determination of a far-seeing physician to open new paths of approach to obscure areas. His most comprehensive work concerned the relation of abnormalities of the intestinal flora to systemic disorders such as anemia and retardation of growth. I cannot find that they now form an accepted part of present-day medical teaching. I wish his work might be reexamined in the light of present knowledge of the formation of vitamins in the intestinal tract.

In no formal sense was Herter a teacher—certainly not a didactic teacher. Listeners to his lectures could learn how a restlessly inquiring intellect sought approaches to medical problems; they could recognize artistic delight, exhibited in the description of successful attacks which great investigators have made against medical ignorance; they could thrill with excitement when he analyzed the achievements of such masters as Pasteur, Ehrlich and Helmholtz.

He had little of the organizing talent so conspicuous among Abel's gifts. Yet the establishment in 1893 and the subsequent growth of his own laboratory was a unique landmark in the medicine of New York City—indeed of the country. He was one of the most influential of the six original trustees who, in 1901, shaped into the reality and form of the Rockefeller Institute Mr. Rockefeller's determination that something great should be done for medicine; he proposed and achieved the establishment of the Rockefeller Hospital as a laboratory for the scientific study of human disease. He endowed two permanent lectureships—one at Hopkins the other at the Bellevue School—designed to give medical students the opportunity of acquaintance with the personalities of the creative investigators in the sciences of medicine.

Essentially an artist, with the artist's never-ending quest for beauty and perfection, he was often intensely dissatisfied with his own efforts but was never-failing in sympathetic helpfulness to young men and in generous and discriminating appreciation of the successes of others.

Professor Hemmeter, writing about Herter in 1911, cites Wilhelm Ostwald's differentiation of investigators into two categories. One he called classicists, conceived as being persistent, thorough, penetrating, objective workers who exhaust or aim to exhaust their subjects; those words seem to me to describe Abel. The other category Ostwald called romanticists, men who are quick, subjective, less thorough, their minds occupied with a multiplicity of problems. They produce and formulate ideas; are possessed of great wealth of thought and superabundance of conceptions. If Herter is to be classified, I would put him into this latter group.

This anniversary marks a milestone in the life of a publication which has been a tremendous factor in the advance of biochemical science here and abroad. We take pride in its quality, the soundness of its growth and its present magnitude and influence. I am inviting your especial tribute to the contrasting but complementary qualities of greatness of the two pharmacologists whose effort brought the Journal into being and whose characters have been projected into it throughout the fifty years of its life.

DEVELOPMENT OF INTERNATIONAL ORGANIZATION OF BIOCHEMISTRY

ELMER STOTZ

From the University of Rochester School of Medicine and Dentistry, Rochester, New York

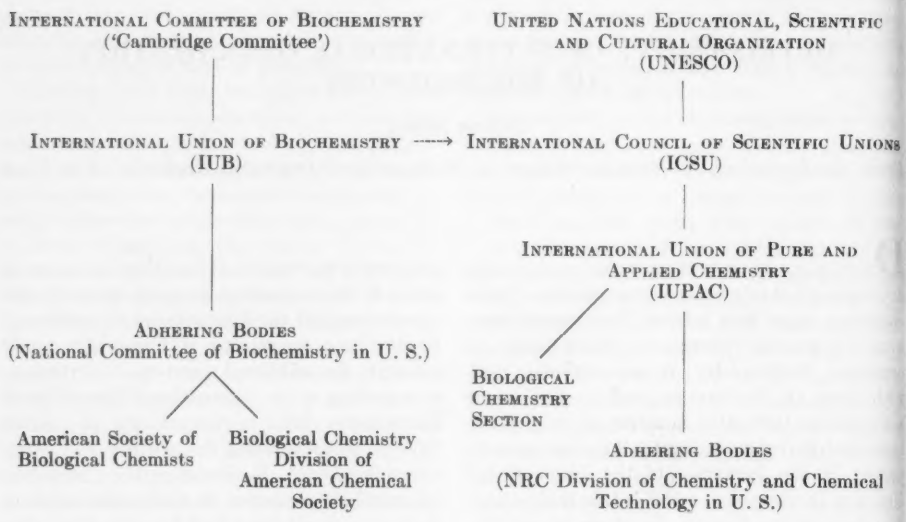
DR. VAN SLYKE, in inviting me to speak on the development of international organization of biochemistry, must have believed that I could draw some comparison between the development of organized biochemistry at our national level with that at the international level. I must acknowledge that other members of our Society have contributed more directly than I to separate phases in the formation of the International Union of Biochemistry, but it is true that as past Secretary of the Society, Secretary of our National Committee, and as Treasurer of the International Union of Biochemistry, I have been in the cross-fire of events during most of the short history of the Union.

In contrast to the annual reunion and cooperation of biochemists made possible in the United States by the formation of this Society 50 years ago, the First International Congress of Biochemistry was held in Cambridge, England, only in the summer of 1949, a meeting attended by 1,741 persons from 42 countries. At the concluding session of this Congress, those in attendance voted for creation of an International Union of Biochemistry, and established an International Committee, not only to organize further congresses, but to approach the International Council of Scientific Unions toward admission of a Union of Biochemistry. Sir Charles Harington was chairman of this Committee, and Dr. John Edsall and Dr. Robert Jackson represented the United States. At the time there existed an International Union of Pure and Applied Chemistry (IUPAC) within the International Council of Scientific Unions (ICSU), and it is probable that discussions were already under way to establish a Section of Biological Chemistry within the chemical Union, since already in the Fall of 1949 such a Section was established.

The first President of the Biological Chemistry Section of the International Union of Pure and Applied Chemistry was Professor Tiselius, and Dr. John Edsall and Dr. Murray Luck were members of the Section Committee. A number of places in this committee were left open with the expectation that the International Committee of Biochemistry constituted at Cambridge would nominate the additional members. Nevertheless, at a meeting of the International Committee of Biochemistry held in Copenhagen in August 1950, at which meeting this country was represented by Dr. A. B. Hastings, the Committee reiterated its resolution to make application to the International Council of Scientific Unions for an independent Union of Biochemistry, and framed the first Statutes of such a Union. After circulating these Statutes, they were revised for presentation to the Executive Committee of the International Council of Scientific Unions in October 1951 at Washington. During this period, there existed mutual understanding and cooperation between the biochemical groups in the International Union of Pure and Applied Chemistry and the International Committee. There were members common to both organizations, and when in 1951 Sir Charles Dodds assumed the Presidency of the Biological Chemistry Section of the International Union of Pure and Applied Chemistry, it was with the understanding that he favored an independent Union of Biochemistry and would continue to work for its establishment.

The result of the Washington meeting was principally a recommendation that there be further discussion between the two biochemical groups at the Second International Congress of Biochemistry in Paris in July 1952. The International Committee was again invited to nominate five members to the Section Committee of the International Union of Pure and Applied Chemistry. Thus it seemed desirable to conduct a poll of biochemists in this country and in Great Britain, seeking their opinions as to whether to continue to press for an independent International Union of Biochemistry or to join the existing chemical Union. Of course it was easier to vote for independence and not to be concerned with the proba-

TABLE 1


INTERNATIONAL COMMITTEE OF BIOCHEMISTRY
('Cambridge Committee')
UNITED NATIONS EDUCATIONAL, SCIENTIFIC AND CULTURAL ORGANIZATION
(UNESCO)
INTERNATIONAL UNION OF BIOCHEMISTRY
(IUB)
INTERNATIONAL COUNCIL OF SCIENTIFIC UNIONS
(ICSU)
INTERNATIONAL UNION OF PURE AND APPLIED CHEMISTRY
(IUPAC)
ADHERING BODIES
(National Committee of Biochemistry in U. S.)
BIOLOGICAL CHEMISTRY SECTION
American Society of Biological Chemists
Biological Chemistry Division of American Chemical Society
ADHERING BODIES
(NRC Division of Chemistry and Chemical Technology in U. S.)

bility of admission to the International Council of Scientific Unions and the technicalities of administration if such admission should occur. Thus the representatives of Great Britain and other countries, with Dr. Severo Ochoa and the late Dr. Erwin Brand representing the United States, went to Paris with a positive vote of their countries' biochemists in favor of an independent Union of Biochemistry. On the other hand, the delegates of our National Research Council, which is the 'adhering body' of the International Union of Pure and Applied Chemistry, attended with the NRC resolution that it was "opposed to formation of separate International Unions covering only parts of chemistry".

At the Paris meeting a feeling was expressed that biochemists were not always adequately represented in their national chemical organizations. Once again the International Committee of Biochemistry renewed its determination to gain entrance to the International Council of Scientific Unions as an independent Union. At this time Sir Charles Harington retired as chairman of the International Committee of Biochemistry, and was succeeded by Professor Marcel Florkin of Belgium.

The new application of the International Committee of Biochemistry was presented to the International Council of Scientific Unions at Amsterdam in the Fall of 1952. At this time a rather serious obstacle was introduced, namely, that a biochemical Union had first to be formally constituted before application, necessitating 'adhering bodies' in at least 12 countries. Furthermore, a 'straw vote' was taken on the provisional acceptance of an International Union of Biochemistry, and the vote was unfavorable.

To a less vigorous group than the International Committee of Biochemistry this would have been a telling blow, but within the ensuing months the appropriate adhering bodies were established and the International Union of Biochemistry with an 'Interim Council' was established on March 1, 1953. The International Committee of Biochemistry became the 'Interim Council', and Dr. Severo Ochoa and Dr. J. S. Fruton were U. S. representatives in the Interim Council. The adhering body in this country to the new Union is the National Committee of Biochemistry, appointed jointly by our Society and the Biological Chemistry Section of the American Chemical Society. The present composition of the National Committee is Dr. V. du Vigneaud, chairman, Dr. R. H. Barnes, Dr. R. D. Coghill, Dr. S. Gurin, Dr. C. G. King, Dr. W. M. Stanley and Dr. E. Stotz.

Remaining in the formal establishment of the new Union was a meeting at which the Statutes of the Union could be finally approved, a more permanent Council formed and officers elected. For this purpose a grant of $3,000 from UNESCO to the Interim Council was made, and with the aid of our National Science Foundation Dr.

Ochoa and I (replacing Dr. Fruton) attended the London meeting in early January 1955. At this meeting the Statutes were formally adopted by representatives from 15 countries, and a Council of 20 members and the officers were elected. The Council members and officers are indicated in table 2. A new application for admission of the Union to the International Council of Scientific Unions was prepared with the aid of Dr. Ronald Fraser, Administrative Secretary of the International Council of Scientific Unions, and with the cooperation of representatives of related Unions.

By the time of the second Council and General Assembly meeting of the Union in Brussels, on the occasion of the Third International Congress of Biochemistry in August 1955, five other countries had made application for membership in the International Union of Biochemistry. These are listed in table 3. It was also made known that with the particular aid and support of Sir Charles Dodds and Dr. Murray Luck, the International Union of Pure and Applied Chemistry at its Zurich meeting went on record that 'it would not oppose' entrance of the new International Union of Biochemistry into the International Council of Scientific Unions. It was further proposed that there be a Coordination Committee to prevent duplication and establish cooperation between the two biochemical organizations. This proposal was welcomed by the International Union of Biochemistry, and the new Coordination Committee held a preliminary meeting in Brussels. Members from the International Union of Pure and Applied Chemistry are Professors Luck, Fromageot and Stoll, and from the International Union of Biochemistry its officers are Florkin, Thompson and Stotz. There is every reason to believe that cordial relations and cooperation will be continued between the two organizations and among the biochemists concerned.

At the Oslo meeting of the Executive Committee of the International Council of Scientific Unions, the petition of the International Union of Biochemistry was again presented, and this time success was achieved. Much credit for this final outcome is due to Sir Charles Harington and Professor Marcel Florkin for their devoted and untiring efforts in reaching the final goal.

The aims of the International Union of Biochemistry are simple and broad, namely: *a*) to encourage the continuance of a series of International Congresses of Biochemistry; *b*) to promote international coordination of research, discussion and publication; *c*) to organize a permanent cooperation between the societies representing biochemistry in the adhering countries; and *d*) to contribute to the advancement of biochemistry in all its international aspects.

TABLE 2. FIRST GENERAL ASSEMBLY AND COUNCIL

(London, January 1955)

Great Britain	*Japan*
Sir Rudolph Peters	S. Akabori
R. H. S. Thompson (Sec.)	*Australia*
Sir Charles Harington (Hon. Member)	A. H. Ennor
France	*Austria*
J. E. Courtois	O. Hoffmann-Ostenhof
J. Roche	*Denmark*
USSR	K. Linderstrøm-Lang
V. A. Engelhardt	*Norway*
A. I. Oparine	R. Nicolaysen
Germany	*Sweden*
K. Felix	E. Hammarsten
K. Lohmann	*Netherlands*
U. S.	H. G. K. Westenbrink
S. Ochoa	*Finland*
E. Stotz (Treas.)	A. Virtanen
Belgium	*Italy*
M. Florkin (Pres.)	A. Rossi-Fanelli

TABLE 3. SECOND GENERAL ASSEMBLY

(Brussels, August 1955)

(*Additional member countries admitted*)

Canada	*Czechoslovakia*
R. J. Rossiter	J. Horejsi
Hungary	*Poland*
I. Banga	J. Heller
Spain	
D. A. Santos Ruiz	

Toward these ends, the International Union is already engaged in plans for the Fourth International Congress of Biochemistry to be held in Vienna in 1958, is sponsoring symposia in Japan and Russia in 1957 and will establish an International Commission on Enzymes. In its work the Union, through its national delegates, is responsive to our National Committee, which in turn represents our two national biochemical groups. Biochemistry, not merely a branch of chemistry or physiology, has thus reached the same status among the scientific disciplines at the international level as that accomplished by this Society in our country fifty years ago.

RESOLUTION OF PAST AND PRESENT MEMBERS OF THE EDITORIAL BOARD OF THE JOURNAL OF BIOLOGICAL CHEMISTRY

Presented by DONALD D. VAN SLYKE

On this twentieth year of service of Rudolph J. Anderson as Managing Editor of the Journal of Biological Chemistry *the assembled past and present members of the Editorial Board feel it fitting to express in some degree their feeling of gratitude and admiration for the years of devoted service that Anderson has rendered to the science of biochemistry. He has borne the editorial burden through long years while the ever-increasing number of papers magnified steadily the labor and responsibility of selecting those most entitled to space in the Journal, editing them to conform to its standards of clarity and condensation, and finally supervising their preparation for publication; for the editorial office of this Journal has also been the publication office where proofs were read, errors corrected, and numberless details handled in a way that has never let down the* JBC's *technical excellence, even in times of the greatest pressure. And, not least of all, the management has required skillful handling to maintain a balanced budget in the face of increasing journal size and costs.*

Anderson accepted the Editorship reluctantly, under great pressure from members of the Editorial Board, at a time when there seemed to be literally no other man who would and could assume the burden. He knew well that the reward would be tolerance from those who were satisfied, less than tolerance from those whose demands could not always be met. But, having accepted the task, he never faltered in seeing it through.

For the great service that Anderson has done to our science, and for the fortitude, fairness, dedication and distinction with which that service has been accomplished, a Medal of Honor would be a fitting reward. In lack of authority to strike an official medal, the Members of the Editorial Board who have served with him during these twenty years ask him to accept, as a token of their admiration and affection, a vase with the inscription, "R J A, from his Editorial Board, 1936–56." We hope that it will serve as a symbol and souvenir of the place that he holds in the hearts of those who have had the honor of being his associates, and we wish Rudolph Anderson health and strength to continue his illustrious service to the Journal and to science through years to come.

Atlantic City, April seventeenth, 1956

AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS

Fiftieth Anniversary Symposium: Nucleic Acids[1]

Chairman: HERBERT E. CARTER

OPENING REMARKS BY THE CHAIRMAN

ON BEHALF of the American Society of Biological Chemists it is my happy responsibility to welcome you to this symposium arranged as part of the Golden Jubilee program of the Society. Almost fifty years ago—December 26, 1906 to be exact—a group of 29 chemical biologists met in New York City at the instigation of Professor John J. Abel to consider the founding of a national society of biological chemists. To quote from the discussion of Dr. Abel, "We wish to draw into our society the biological chemists of all departments of biology, including those organic and physical chemists who take a lively interest in our subject. . . . Our common meeting ground should be chemistry as applied to animal or vegetable structures, living or dead. . . ." At that meeting the Articles of Agreement for the "Organization and Provisional Government of the American Society of Biological Chemists" were adopted and officers and council were elected. Subsequently an additional 52 biochemists—making a total of 81—were named as charter members of the new Society by vote of Council. The first and second scientific meetings of the Society were held in 1907 (May and December respectively) and subsequent meetings were held annually. At that first meeting in Washington a pattern was set which still frustrates the society secretary for, although only 26 members attended, some 41 papers were read.

[1] Atlantic City. April 17, 1956.

It is of particular interest to us this morning that two of the original charter members of the Society were Phoebus A. Levene and Walter Jones. The unique contributions of these men to studies of the structure of nucleic acids are known to all of you and are well reviewed in the books which the two men wrote—*Nucleic Acids, Their Chemical Properties and Physiological Conduct* by Jones and *Nucleic Acids* by Levene and Bass. These two treatises afford a most interesting study of the application of brilliant minds to inadequate experimental data in a most difficult field.

At the first meeting of the Society three papers on nucleic acid biochemistry were presented—one each by P. A. Levene, T. B. Johnson and Lafayette B. Mendel. By the fifth meeting in 1910 the tempo had increased and Jones and Levene each contributed two papers.

Subsequent years have witnessed a tremendous expansion in both the Society and the field. The present membership numbers well over 1100 and of the 557 papers on the present program of the Society almost 10% will deal more or less directly with nucleic acid biochemistry.

I am sure that there were no 'squeaky genes' in the chromosomal material responsible for this sound and vigorous growth. Our program this morning, ranging from chemistry to genetics, amply testifies to the versatility of our speakers and the breadth of the nucleic acid field.

VIRUS COMPOSITION AND STRUCTURE—25 YEARS AGO AND NOW

WENDELL M. STANLEY

From the Virus Laboratory, University of California, Berkeley, California

WHEN I WAS INVITED to participate in this Fiftieth Anniversary Symposium and to speak on the subject, "Virus composition and structure —25 years ago and now," I wondered about the suggestion that I cover 25 rather than 50 years. Viruses were first discovered in 1892 and by 1906 several representatives of animal, plant and human viruses were under study. Yet for over 25 years about all that was known about viruses was that many of them were known to exist as agents of disease generally smaller than bacteria and that they could grow or multiply and mutate only within the living cells of specific hosts. During this period viruses were generally regarded as being still smaller bacteria and hence as living organisms. No virus had been purified, concentrated and isolated as such; hence there was no information regarding virus composition and structure. Therefore, the restriction of this discussion to events of the past 25 years will cause no difficulty, but will only serve to emphasize the striking advances which have been made in this field during this time.

Early in the 1930's, as a result of the filtration experiments of Elford (1) and the work of others, evidence was obtained which indicated that the sizes of viruses ranged from about 300 mμ down to about 15 mμ. Thus, the largest viruses appeared to be larger than certain accepted organisms that could be cultivated on artificial media whereas the smallest viruses appeared to be smaller than certain accepted protein molecules, such as those of hemocyanin. There resulted a growing unrest and dissatisfaction with the acceptance of viruses as living organisms for it was argued that all of the metabolic machinery normally associated with a living organism could not be packed into a volume no larger than a single protein molecule. The viruses were soon surrounded with all of the elements of a first-class mystery and arguments concerning their true nature became numerous and quite heated. Some investigators thought that because the basic properties of all viruses were the same, all viruses must be similar in nature; whereas other argued that they differed so much in size they should be divided into three groups, namely, living organisms, non-living chemical molecules and agents representing a new kind of life unfamiliar to us. When a crystalline material possessing all of the properties of tobacco mosaic virus was isolated in 1935 (2), the scientific community, especially that represented by medical investigators, was not ready to accept the crystalline material as the virus. Tobacco mosaic virus was known to be a medium-sized virus and the view persisted that such viruses had to be living organisms and could not be obtained in crystalline form. It was obvious that the most important task at that time was to get the crystalline material characterized as thoroughly as possible and then to secure evidence that the molecules making up the crystals actually did or did not possess virus activity as an intrinsic property. The crystalline material was proved to be a nucleoprotein consisting of 94% protein and 6% of ribonucleic acid and possessing a molecular weight greater than that of any other known protein, namely, 50 million. The individual molecules were found to be 15 mμ in diameter and 300 mμ in length (3). The characteristic nucleoprotein could be isolated from all kinds of mosaic diseased plants including plants whose normal proteins bore no serological relationship to each other. It has never been found in sources other than mosaic-diseased tissues. Destruction of the nucleoprotein by any of several physical and chemical procedures has always resulted in the loss of virus activity. Strains of tobacco mosaic virus have been found to consist of nucleoprotein molecules of identical size and shape but with different amino acid compositions (4). The latter finding represented the first information regarding chemical changes which may accompany the mutation of a virus. Although from time to time there were reports such as *a*) the nucleoprotein could be converted to low molecular weight material by solution in urea without loss of activity and *b*) the nucleo-

protein consisted of aggregates of low molecular weight 'virus' particles, there has been no verifiable experimental evidence during the ensuing 20 years which is contrary to the assumption that virus activity is an intrinsic property of the physical structure 15 by 300 mμ in size. Nevertheless that it might eventually prove possible partially to degrade the molecule with retention of activity was realized for it was stated in 1946 that "It must be recognized that, as with any biologically active material, the possibility always exists that at some future time, perhaps through some new methods of treatment of virus preparations or through new inoculation techniques, virus activity may be found associated with smaller particles" (5). In this connection it may be noted that in 1952 Harris and Knight (6) reported that about 3000 threonine residues per molecule of tobacco mosaic virus could be removed by carboxypeptidase without diminishing the virus activity. However, of the greatest importance is the recent finding of Fraenkel-Conrat (7) that the nucleic acid component of tobacco mosaic virus possesses virus activity. Before discussing this work I should first like to mention the progress which has been made with a variety of other viruses.

Following the isolation of tobacco mosaic virus in the form of a crystallizable nucleoprotein, about a dozen other plant viruses were isolated in the form of characteristic nucleoproteins. Some of these, such as tomato bushy stunt, tobacco necrosis, cucumber mosaic, turnip yellow mosaic and tobacco ringspot, have been obtained in crystalline form. A dozen or so animal and bacterial viruses have also been obtained in highly purified form. All of these purified viruses have proved to be at least as complex as a nucleoprotein and some appear to have lipid and carbohydrate as additional components. Some years ago Knight (8) found that purified influenza virus appeared to have incorporated into the virus a normal host component characteristic of the host in which the virus was produced. Beard and coworkers (9) have recently found a similar situation to exist in the case of their purified fowl erythromyeloblastic disease virus. However, these two examples represent the exceptions from the usual finding that the purified viruses are quite different from the normal components of the host in which they are produced. Although many of the smaller viruses have properties which are compatible with the idea that they are actually nucleoprotein molecules, it should be recognized that some middle-sized viruses, such as the bacterial virus T2, and the larger viruses, such as vaccinia virus, seem to possess external membranes and a degree of internal morphological differentiation which make it impossible to consider them to be molecules in the ordinary sense. However, evidence provided by the bacterial viruses, by the bacterial transforming principle, by the transduction phenomenon and more recently by Fraenkel-Conrat's work on tobacco mosaic virus may serve to focus attention once again on the unity and basic nature of virus activity and to emphasize anew the importance of nucleic acid structure.

Despite the fact that tobacco mosaic virus was obtained in crystalline form in 1935, no virus affecting man or animals had been crystallized until last year. During the ensuing 20 years there was a growing tendency to regard animal viruses as being fundamentally different from plant viruses in spite of growing experimental evidence for similarity. It was, therefore, of considerable importance when Schwerdt and Schaffer (10) of the University of California Virus Laboratory announced last year that they had obtained MEF-1 human poliovirus in crystalline form. Thus the first crystallization of a virus affecting man or animals came just 20 years after the first crystallization of a virus. It is also of interest to note that poliovirus was found to be a nucleoprotein containing about 30% of ribonucleic acid. Thus, although all of the plant viruses contain ribonucleic acid, the bacterial viruses contain deoxyribonucleic acid and the animal viruses may contain one or the other or both types of nucleic acid.

Electron microscopy, the use of radioactive tracers and genetic studies involving bacterial viruses and their hosts have yielded much valuable information. It is now well known that the T2 bacterial virus attaches by its tail to the susceptible *E. coli* cell and that the contents of the viral structure enter the bacterial cell while the outer membrane of the virus remains outside the cell. The entering material which seems to consist largely of deoxyribonucleic acid appears to cause the production of more of itself in subunit form, which eventually is assembled into larger units and covered with protein. When the bacterial cell bursts, a hundred or so bacterial virus particles may be seen. It is also now well-known that infection with two related but genetically different viruses can result in a recombination of the genetic factors and, hence,

that the viruses appear to contain what may be regarded as genes which appear to multiply independently within the bacterial cell. These results stimulated similar work with animal viruses and within the past few years evidence has been obtained by Burnet (11) and by Hirst (12) that a similar phenomenon takes place during the production of influenza virus. Similar results have not yet been produced with the small plant or animal viruses but recent developments in our laboratory indicate that the genetically important component of tobacco mosaic virus is probably the nucleic acid rather than the protein or the nucleoprotein.

It was reported by Cohen and Stanley (13) in 1942 that freshly isolated tobacco mosaic virus nucleic acid was highly asymmetric and possessed a molecular weight of the order of 300,000, then a value by far the largest ever reported for a ribonucleic acid. This material was found to be unstable and to decompose with the formation of asymmetric particles of lower molecular weight. These authors suggested that the nucleic acid molecules, perhaps eight in number, were bound individually along the length of the virus molecule. However, it was not until 1955 that Stahmann and Kaesberg (14) and also Schramm *et al.* (15) provided experimental evidence for localization of the nucleic acid within the virus rods. It was found that occasional rods of tobacco mosaic virus in frozen and dried preparations were fragmented transversely and that the fragments appeared to be bridged by slender threads. A similar appearance was noted following mild alkali treatment. The most definitive evidence was provided in an electron microscope study by Hart (16) who found it possible to degrade the rod under controlled conditions with a detergent. He produced rods of tobacco mosaic virus in which a portion of material was removed from one end, leaving an axially located core about 5 mμ in diameter. Treatment of this preparation with deoxyribonuclease or with trypsin caused no change whereas treatment with ribonuclease caused the extended core to disappear. Hart concluded that the core is ribonucleic acid and that it is axially located within the virus rod. There was no evidence of the core material fraying at the ends into thinner fibrils as might be expected of a multistranded material. Nevertheless, the size and stiffness of the core material is more compatible with a multistranded than with a single stranded nucleic acid. The exact manner in which the nucleic acid is combined with its protein overcoat is still unknown, but there is suggestive evidence that a hole about 4 mμ in diameter exists along the axis of the rod and that the phosphate groupings of the nucleic acid are located about 4 mμ from the axis (17). If this proves correct, it is obvious that there must be an area in which the protein and nucleic acid components overlap (see fig. 1).

Considerable work has been done on the amino acid content of several purified virus preparations (see table 1). However, no new amino acid or no amino acid in what might be considered unusual amounts has been encountered and there is, therefore, nothing sufficiently unusual about the amino acid content to distinguish the viruses. Perhaps it should be noted that the viruses do not contain large amounts of basic amino acids and thus they differ from the protamines and histones. It was found quite early that treatment with alkali (2), sodium dodecyl sulfate (24) or urea (25) caused tobacco mosaic virus to break up with the formation of protein subunits of low molecular weight. Later Schramm (26) noted that under certain conditions the protein subunits in alkali-treated virus preparations could be caused to aggregate into rod shaped but non-infectious particles. Although the chemical subunit based on the 3,000 threonine residues released by carboxypeptidase treatment and on the cysteine content would be about 17,000, a value fairly consistent with x-ray evidence, the material possessing the ability to aggregate to form rods seems to have a molecular weight of about 100,000. Detailed structural studies involving analyses for end-groups, amino acid content and sequences of the amino acids in the chemical subunit of tobacco mosaic virus are under way. Although threonine is the C-terminal amino acid, the N-terminal end appears to be bound in a ring structure. At present about 10% of the 145—residue chain of the tobacco mosaic virus subunit is known but it may be some years before the actual structure is determined. It is a matter of interest that Takahashi and Ishii (27) have isolated an abnormal non-virus protein from mosaic-infected plants which possesses the ability of aggregating to form non-infectious rods similar in appearance to those of the virus and of the aggregates formed from the virus protein subunits. It is also interesting to note that partial aggregation of the virus protein subunits or of the abnormal protein yields products which appear as washers about 15 mμ in diameter with a central hole about 4 mμ in diameter. Many attempts have been made to

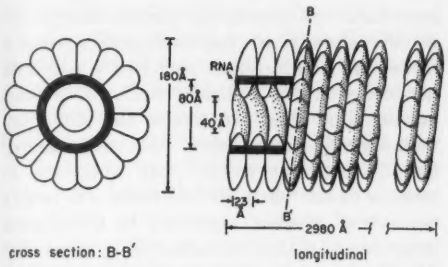


FIG. 1. Schematic representation of a tentative structure for tobacco mosaic virus based upon discussions with members of the Virus Laboratory and others and upon the following experimental evidence. Obviously, there is great over-simplification.

Evidence exists from both electron microscopy and from x-ray analysis (18) to show that the center-to-center separation of the rods of TMV (tobacco mosaic virus) is 150 Å, when the rods are packed in either a two-dimensional or three-dimensional array. However, recent study (17) of the fine structure of the x-ray patterns of crystalline TMV, indicates strongly that the external contour of the rods is deeply grooved. This would account for the observation from radial distribution functions (18a) that the total diameter of the rods is as great as 180 Å, but that, upon intermeshing, screw-wise, the center-to-center separation of the rods is only 150 Å. There is some support for this conclusion from electron microscopy, since it is universally found that the apparent diameter of single rods is greater than the average diameter of several rods packed closely side by side.

The length of the rods has been precisely established by electron microscopy (3) and has been found to be 2980 ± 10 Å. Preparations can be made in which over 90% of the TMV particles are either of this length or are a small multiple of this length (dimers or trimers).

There exists some discrepancy concerning the external contour of the rods. Williams (19) found that short sections of the rods have a six-sided contour in the form of a hexagon. The x-ray evidence for 16⅓ subunits per turn in the intact virus makes a hexagonal external contour seem unlikely. However, the electron microscopic observations were made on very short fragments of the rods, and it is possible that distortion of these fragments takes place upon drying.

X-ray studies have demonstrated that the arrangement of the protein subunits was quite regular (18) and in the form of a helix (20). The molecular weight of the chemical subunit is believed to be about 18,000 (6). The pitch of the helix is 23 Å, and the arrangement is such there must be $3n + 1$ subunits in three turns of the helix. Although the preliminary determinations (20, 21) gave 10 or 12 as the most likely value for n, the most recent work with mercury substituted TMV yields a value of $n = 16$ (22). At present, then, there appear to be 49 subunits in a length of 69 Å along the rod. The best fit with the chemical data, and with the molecular weight of the particle (23), is obtained by assuming that each crystallographic subunit is also a chemical subunit.

The localization of the RNA (ribonucleic acid) in the virus rods has been secured by both electron microscopy and by determination of electron radial distribution functions. The electron microscopic results have been obtained by photographing particles whose ends have been partially dissolved by treatment with sodium dodecyl sulfate (16). After such treatment a rather well-integrated core, of about 40 Å diameter, is seen to protrude along the axis of the residual virus particle, and this core is removable with RNase. The localization of the RNA has recently been determined by comparing the radial distribution of electron density of native TMV with that of the polymerized protein fraction (18a). The difference in the two distributions presumably locates the x-ray scattering electrons in the RNA. These x-ray results indicate that the RNA, presumably the phosphate groups, is localized some 40 Å from the axis of the rod at a distance about half way between the axis and the mean outer surfaces. Such a localization demands that there be some protein between the RNA and the axis of the rod. This makes reconstitution of the nucleoprotein appear quite difficult unless it is assumed that the protein units entering into the reconstitution are so shaped that the RNA can be embedded within a crevice or chink in the protein units (22). There is no discrepancy between the electron microscopic and the x-ray results as to the localization of the RNA.

The x-ray results (18a) indicate that the virus rod has a hole along its axis of radius about 20 Å. There is no evidence from electron microscopy bearing on this except for the fact that washer-like structures are seen when the virus protein is partially polymerized in the absence of nucleic acid.

The electron microscopic evidence of the rather large, stiff core of RNA, with no sign of an extended uncoiling (which a single strand might do), rather suggests that a multistranded model is possible. The answer would be clearer if the electron microscope pictures would show evidence of fraying of the core into multistrands.

copolymerize the nucleic acid and protein components of tobacco mosaic virus but significant success was not obtained until last year. Fraenkel-Conrat and Williams (28) reported that protein derived from the virus by careful treatment with alkali would combine stoichiometrically with ribonucleic acid obtained from the virus by careful treatment with sodium dodecyl sulfate to yield nucleoprotein rods containing about 6% nucleic acid and 94% protein and possessing virus activity. There is evidence that the nucleic acid in the reconstituted virus rods is located along the central axis as in the normal virus rod.

TABLE 1. AMINO ACID CONTENT OF SOME HIGHLY PURIFIED PREPARATIONS OF VIRUSES AND RELATED MATERIALS

Constituent	Virus							
	TMV*	CV4	BSV	T3	PR8	LEE	NAP	SPV
	Grams amino acid residue/100 gm virus							
Alanine	4.1	4.9	6.0	3.4	2.0	2.1		2.5
Arginine	8.8	8.3	5.7	2.8	4.5	3.6	3.5	6.2
Aspartic acid	11.7	11.3	8.9	4.4	6.4	6.4	5.4	9.5
Cysteine	0.6	0.0	0.6†					
Cystine	0.0	0.0	‡					5.4
Glutamic acid	9.9	5.7	4.4	4.1	6.8	5.5	5.4	10.2
Glycine§	1.4	1.1	3.7	3.3	1.9	2.2	1.4	2.4
Histidine	0.0	0.0	1.1	0.7	1.2	1.3	0.7	1.7
Isoleucine	5.7	4.0	5.8	1.8	3.5	3.6	2.8	3.6
Leucine	8.0	8.1	9.2	3.7	4.6	4.7	3.7	6.3
Lysine	1.3	2.1	3.7	2.4	3.2	4.1	2.2	6.2
Methionine	0.0	0.0	1.8	0.7	2.0	1.9	1.0	1.9
Phenylalanine	7.5	8.7	4.2	1.4	3.3	3.0	3.2	4.1
Proline	4.9	4.8	2.4	1.5	2.2	2.3	2.4	4.6
Serine	6.0	7.8	5.1	1.1	1.8	1.8	1.7	3.3
Threonine	8.4	5.9	8.1	2.0	3.1	3.4	3.2	4.4
Tryptophan	1.9	0.5	0.5		1.0	0.6	0.6	0.9
Tyrosine	3.4	3.3	4.1	1.6	2.8	1.9	2.0	5.3
Valine	7.8	7.5	10.3	2.4	2.9	2.7	2.7	4.6
Total	91.4	84.0	85.6	37.3	53.2	51.1	41.9	83.1
Percent nucleic acid	6	6	17	50	5	5		9

* Abbreviations stand for the following: TMV, tobacco mosaic virus; CV4, cucumber virus 4; BSV, tomato bushy stunt virus; T3, T3 bacteriophage; PR8, strain of influenza A virus; Lee, strain of influenza B virus; NAP, normal allantoic particles; SPV, Shope papilloma virus.

† BS gives + nitroprusside test for S and hence cysteine assumed present; the above amount was calculated from total S-methionine sulfur. A recent determination of cysteine (Fraenkel-Conrat, personal communication) gave a value of 0.8 percent cysteine residues.

‡ Blank areas indicate that no analysis has been made for that constituent.

§ Glycine values were not corrected for possible contributions arising from the decomposition of purines during hydrolysis of the viruses. However, this is probably negligible in all except BSV and T3.

Table from C. A. KNIGHT. *Advances in Virus Research*, New York: Academic Press, vol. II, 1954.

The material in the reconstituted preparation is heterodisperse in length, with most of the rods being less than 300 mμ in length, some being 300 mμ in length and a few being longer. However, a high degree of correlation between the presence of rods of length $\geq$ 300 mμ and the infectivity has been noted.

Recently Fraenkel-Conrat (7) has prepared what might be called a hybrid virus by combining the nucleic acid derived from the ribgrass strain of tobacco mosaic virus with the protein from the ordinary strain of tobacco mosaic virus. Although no structural differences have as yet been found between the nucleic acids derived from these two strains, the protein components do differ since the ribgrass virus protein contains methionine and histidine, amino acids which do not occur in the ordinary strain of tobacco mosaic virus. The two strains also differ immunologically and, when the reconstituted hybrid virus was treated with antiserum to tobacco mosaic virus, its infectivity was largely neutralized whereas antiserum to the ribgrass strain caused little or no reduction in infectivity. The disease produced on inoculation of Turkish tobacco plants with the hybrid virus was that characteristic of the ribgrass strain. Thus, the reconstituted hybrid virus resembled the ordinary strain in its immunological properties but the ribgrass strain in its disease-producing capacity. The progeny virus produced on inoculation of the hybrid was found to contain methionine and histidine like the ribgrass virus protein from which the nucleic acid of the inoculum was derived and unlike the protein overcoat of the infecting virus particles. These results demonstrate that the ribonucleic acid core of the virus carries the genetic message.

The importance of the nucleic acid is also indicated by recent work of Hart and Smith (29) who have combined tobacco mosaic virus protein subunits with various kinds of nucleic acids including the synthetic polynucleotides about which you will hear later on this morning. Although different kinds of nucleic acids including several synthetic polynucleotides combined with tobacco mosaic virus protein to yield the characteristic rod-like structures 15 mμ in diameter, these were not found to possess virus activity on any plants so far tested. Whether or not they will reproduce in some as yet untested cell is, of course, not known. They could represent a virus in search of a host.

Further evidence regarding the importance of the nucleic acid was obtained recently when it was found that certain preparations of virus nucleic acid carried a residual infectivity which differed in its stability from that characteristic of the regular rod of tobacco mosaic virus. This residual infectivity was found only in very fresh preparations and disappeared irreversibly when the preparations were permitted to stand at room temperature or were subjected to the action of ribonuclease. It has been concluded that this virus activity is a property of the nucleic acid itself and that the role of the protein is that of a stabilizer which also determines the immunological properties and perhaps, to a large extent,

the host specificity of the virus. It would appear, therefore, that except for the separation of the protein overcoat outside the cell in the case of the bacterial viruses, the process of infection and reproduction of the plant viruses may be similar to that of the bacterial viruses and that, in view of the many distinct strains of tobacco mosaic virus, one should anticipate genetic recombination within such strains. Needless to say, the groundwork which has been laid in the chemical studies on tobacco mosaic virus and its strains which has provided knowledge such as the fact that methionine and histidine occur in one strain and are absent in another strain should prove of the greatest value in such studies.

The discovery that several hundred bits of a special protein of molecular weight of the order of 100,000 can be caused to assemble around, say, 12–16 strands of ribonucleic acid with the formation of a very regular structure 15 mμ in diameter and 100 to 300 mμ in length, and that some of these structures then possess the ability to bring about their reproduction within certain living cells is most important. Equally important is the discovery that the ribonucleic acid appears to carry the genetic message and that apparently, under certain conditions, the ribonucleic acid can cause infection without its protein overcoat. Although work on the elucidation of the protein structure characteristic of certain viruses should proceed because of the potential value in connection with the production of polypeptide antigens for prophylactic vaccines, it is obvious that the really important problem is posed by the structural characteristics of the nucleic acids. It would appear that a ribonucleic acid can direct the course of intracellular events that result not only in its own replication but also the production of a characteristic protein with which it eventually combines for its own preservation. The fact that a great array of viruses and virus strains are known and that many of these have been obtained in a highly purified form is extremely fortunate for these provide a source for a great variety of genetically distinct materials from which essentially pure nucleic acids may be obtained. The elucidation of the nature of the structural differences that exist between these different nucleic acids that are responsible for their different biological activities is one of the most important problems facing scientists today.

The great development of tissue or cell culture techniques and special activity with the poliomyviruses and with certain neurotropic and respiratory viruses during the past few years has resulted in the discovery of dozens, if not hundreds, of new viruses, most of which are still unrelated to recognized diseases. One cannot help but wonder, in view of the known properties of the bacterial viruses, this recent work wth tobacco mosaic virus and the known plasticity of viruses generally, how many different nucleoproteins or more probably nucleic acids may be replicating within our cells at rates varying from 1 to a 100 or more per cell division and with varying degrees of intimacy with the genetic apparatus of the cell. It may prove necessary to revise the generally accepted definition of a virus as an infectious agent to include situations in which a replicating structure does not appear infectious because normally it never leaves the cell and is duplicated only once during each cell division. Such a nucleoprotein or nucleic acid might at times appear to be a part of the chromosomal material of the cell but be subject to chemical or physical stimulation or shock which could cause it to mutate, separate and act as an independent functional unit with the further possibility of a great increase in the rate of replication. The requirement that a replicating structure, if it is to be regarded as a virus, must have at one time entered the cell from without, that is to say, it is not actually a part of 'self,' still seems to be a reasonable one if we are to avoid utter confusion. However, we may have acquired some of the nucleoproteins or nucleic acids replicating in our cells years or even generations ago and it could prove rather difficult to demonstrate experimentally an event that might have occurred generations ago. Here the question of 'self' or 'not self' as well as that of 'essentiality' may become of importance. Certainly the research opportunities which are provided by the viruses, by virtue of their ability to enter a living cell, to partake and actually largely to direct the metabolism of the cell, following which they can be separated and studied once again are indeed quite wonderful. Twenty-five years have seen the viruses rise from the unknown to provide us with perhaps the most powerful weapon now known to probe that abnormality called cancer and that greatest of all mysteries, life itself.

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PROGRESS IN DEVELOPING CHEMICAL CONCEPTS OF GENETIC PHENOMENA

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FIFTY YEARS AGO, when the birthday whose anniversary we are celebrating occurred, the science of genetics was also in its infancy, and its future was by no means assured. The concept of the gene as a material particle was not yet developed —in fact, the word 'gene' had not been invented —and to some biologists the Mendelian preoccupation with ratios seemed like a form of numerology. In that year, T. H. Morgan himself wrote an article which was highly critical of the Mendelian position. Yet within three years Morgan was to begin the experiments which not only confirmed the essential principles of Mendelism, but elucidated the mechanism of their operation and led to the recognition of the chromosomes as the chief bearers of heredity in plants and animals. Today, we regard Mendelian genetics as furnishing not only an adequate account of the facts of inheritance, but also of evolution. Genetics thus forms the theoretical backbone of modern biology.

Properties of Genes. An essential feature of Mendelian genetics is its particulate nature. The particulate character of the genetic material is shown in breeding experiments, which reveal that the sexual process is simply an elaborate lottery, the primary function of which is to produce new random combinations of the genetic particles. In addition, these experiments reveal two fundamental properties of the genes: *1*) they are self-reproducing and *2*) they are mutable. By 'self-reproducing' we mean that genes arise only from pre-existing genes. In other words, the gene has some essential function to perform in the production of more genes like itself. This property is not unfamiliar to biochemists. Trypsin is self-reproducing in the presence of trypsinogen, and so is acetic acid in the presence of ethyl acetate.

There is a real difference between gene reproduction and the reproduction of trypsin and acetic acid, however. Genes occasionally undergo changes, or mutations, which are recognized by their biological effects. When the mutated gene reproduces, it reproduces the *mutant* type. Nothing like this is known in simple chemical systems, and, indeed, this must be a rare property in nature, because a substance which is self-reproducing and mutable comes close to being alive.

Another important characteristic of the genes is demonstrated in experiments in which they are induced to mutate by treatment with ionizing radiations. It is found that the yield of mutations is proportional to the number of ionizations per unit volume of tissue, and, further, that there is apparently no threshold level of ionization below which mutations are not induced. This means that a single ionization is sufficient to induce a mutation, and it suggests that the gene is a single molecule. Unlike ordinary chemical systems, the living cell thus appears to depend on the integrity of individual molecules. When one compares the magnitude of a single ionization with the magnitude of the effect produced—i.e. death or permanent alteration of all the descendants of the mutated cell—it becomes evident that damage done to the genic material is amplified to an extraordinary degree. This result suggests that the role of the gene is a catalytic one, but in order to explain the immense amplification, it is necessary to assume that the gene functions, not as an ordinary enzyme, but as a catalyst for the production of enzymes.

Biochemical Role of Genes. This notion goes back at least to 1917 (1), but techniques for exploring it have been available only in recent years. Modern studies on the biochemical effects of mutations support the view that the genes have a role in enzyme systhesis. In the last fifteen years, hundreds of mutations have been recorded—chiefly, but not entirely, in microorganisms—the effect of which is to produce specific biochemical lesions in the organism. These lesions result from the abolition of normal chemical reactions in the cell. They are usually expressed by the acquisition of a nutritional requirement, or by the abnormal accumulation of some metabolite. In a number of cases it has been shown by direct methods that the metabolic block is accompanied by a deficiency of the corresponding enzyme (table 1). The presence of an orthodox chromosomal system of inheritance in Neurospora makes it possible to state that the

TABLE 1. PARTIAL LIST OF MUTATION-INDUCED ENZYMATIC DEFICIENCIES

Enzyme	Authors
Neurospora	
Argininosuccinate splitting enzyme	Fincham & Boylen (2)
Cystathionases I and II	Fischer (3)
Cytochromes a and c	Tissieres & Mitchell (4)
Dihydroxy acid dehydrase	Myers & Adelberg (5)
Glutamic dehydrogenase	Fincham (6)
Nitrate reductase	Silver & McElroy (7)
Tryptophane desmolase (synthetase)	Mitchell & Lein (8)
E. coli	
Acetylornithine deacetylase	Vogel (9)
5-Dehydroshikimic reductase	Davis (10)
5-Dehydroquinase	Davis (10)
Diaminopimelic decarboxylase	Dewey & Work (11)
Pantothenate synthesizing enzyme	Maas & Davis (12)
Transaminase B	Rudman & Meister (13)

enzymatic deficiencies result from mutations of chromosomal genes in this organism, but there is little doubt that the bacterial mutations are fundamentally similar to those of Neurospora. It should be noted that the cytochrome system of Neurospora is also under the control of a self-duplicating cytoplasmic agent (14; see also 15).

The evidence of biochemical genetics thus confirms the idea that the genes function in the production of enzymes. Furthermore, there is a strong indication that the role of the genes may be a direct one, since in the majority of mutants the total effect of the mutation can be accounted for by the loss of a single biochemical reaction (16, 17). In other words, it appears that each gene functions in the synthesis of only one enzyme. This conclusion is contrary to the belief, based on morphological studies, that single gene mutations would be found to produce multiple biochemical lesions. The evidence is all the more impressive in the apparently exceptional cases where the mutation results in the acquisition of two or more nutritional requirements simultaneously. It is commonly found in these cases that the multiple requirement actually stems from the loss of a single reaction or enzyme (5, 13, 18–20).

In attempting to assess the meaning of this simple relationship between mutations and enzymes, it is difficult to escape the conclusion that a structural correspondence of some kind exists between the gene and the enzyme it

TABLE 2. MUTATION-INDUCED QUALITATIVE ALTERATIONS OF PROTEINS

Protein	Organism	Property	Authors
Hemoglobin	Man	Electrophoretic mobility	Pauling *et al.* (21)
β-Lactoglobulin	Cattle	Electrophoretic mobility	Aschaffenburg & Drewry (22)
Pantothenate synthesizing enzyme	E. coli	Thermostability	Maas & Davis (12)
Tyrosinase	Neurospora	Thermostability	Horowitz & Fling (23)

controls. The structure, or some specific feature of the structure, of the gene is represented in the structure of the enzyme. This much seems to be required by logical necessity. What follows is merely a guess. The guess is that the gene carries information relating to the composition of the protein molecule. More specifically, it carries instructions regarding the ordering of the amino acids. This is the current form of the template theory of gene action. The role it assigns to the gene is an appropriate one, since it seems evident in any case that the synthesis of specific protein molecules requires a model or a set of instructions which is characteristic for the species, which is automatically transmitted from generation to generation, and which is constant, yet capable of evolutionary change. The only cellular components which are known to fulfill these conditions are the genes.

Structural Mutations of Proteins. If this theory is correct, then it should be possible to obtain mutations that result not merely in quantitative changes in enzyme activity, but also in qualitative changes in enzyme structure. These should be detectable as alterations in measurable properties of the enzyme. Besides being a necessary consequence of the template theory, such modifications could provide its strongest confirmation.

The search for such examples is just beginning. I know of only four cases in which it can be safely concluded that a soluble protein has been genetically modified (table 2). In two of these cases (hemoglobin and tyrosinase) it has been shown that a single gene is involved, and this is likely in a third (lactoglobulin). In regard to the Neurospora enzyme, we have been interested in finding out whether or not other properties of the enzyme are altered along with its thermostability. A number of functional characteristics of the two forms of the enzyme have therefore been measured, including Michaelis constants for two substrates and relative turnover rates. No differences have been found, except in the related property

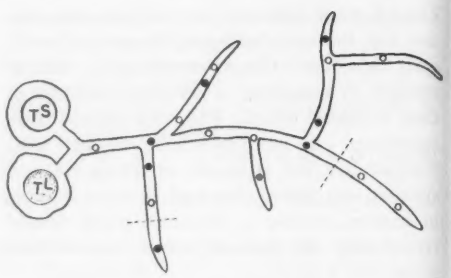

FIG. 1. The heterocaryon is formed by fusion of hyphae from two genetically pure (homocaryotic) strains. It can be resolved into the constituent homocaryotic strains by cutting off hyphal tips (*dotted lines*), some of which contain only one kind of nucleus. Within the heterocaryon, the nuclei share a common cytoplasm. Diagrammatic.

of activation energy of thermal inactivation (24). These findings indicate that genetic control of enzyme structure may be exceedingly fine-grained, permitting the separate alteration of individual features of the structure.

The discovery of the existence of structural mutations is certainly gratifying, but it should be pointed out that modification of a single property of a protein molecule is not a sufficient test of the template hypothesis. If the hypothesis is correct, all of the specific properties of the protein should be susceptible of genetic alteration. It should be possible to produce a series of structurally modified proteins by different mutations of the same gene. This has not yet been accomplished.

In the meantime, further study of the existing cases may throw light on the mechanism of gene action. Recently, we have used the inherited thermostability difference in Neurospora tyrosinase in an experiment designed to test whether synthesis of the enzyme is mediated by self-reproducing cytoplasmic particles. A heterocaryon was constituted, some of whose nuclei carried the gene for thermostability of the enzyme, and some its allele for thermolability (fig. 1). The heterocaryon produces both forms of the enzyme. The heterocaryon was resolved by cutting off the tips of the hyphae; occasionally, such a hyphal tip is homocaryotic, although its cytoplasm is continuous with that of the rest of the culture. Cultures were established from these isolates and the thermostability of their tyrosinase determined. In all cases examined so far, the homocaryotic cultures produce one kind of

tyrosinase, and this in accordance with the genetic constitution of the nucleus (25). This experiment has thus failed to detect the presence of a self-duplicating cytoplasmic agent.

Chemical Nature of Genes. We thus have three basic genetic phenomena which require interpretation in chemical terms: gene reproduction, gene mutation and gene action. The solutions of these problems will depend ultimately on knowledge of the chemical nature of the genes. What do we know about the structure of genes?

Since DNA (desoxypentosenucleic acid) is a prominent constituent of chromosomes and is rarely found outside the nucleus, it has for a long time had a conspicuous place in speculations on the chemical structure of the genetic material. A direct test of the idea that the genes contain DNA—that is, by direct isolation and identification—has never been carried out in any animal or higher plant, because there is no known test which would enable one to recognize the gene once it has been removed from the cell. The contrivance of such a test is a challenging problem whose solution would have consequences far beyond that of this immediate problem, since it would enable us to compare the genetic constitutions of cells which cannot be subjected to the usual kind of genetic analysis. For example, the genes of liver tissue could be compared with those of brain, or those of normal cells with malignant ones.

The closest one has come to a direct chemical analysis of the genetic material of higher organisms is by study of the action spectrum of mutation. A careful analysis of the effectiveness of nine different wavelengths in the ultraviolet in causing mutations in corn has been made by Stadler and Uber (26). It was found that the action spectrum parallels the absorption spectrum of nucleic acid in the tested region.

The situation is different in the microorganisms. In the case of certain bacteria and viruses it has been possible to obtain direct evidence of the nature of the genetic material. Thus, it has been known for some time that material with gene-like properties can be extracted from heat-killed cells of pneumococcus. This material has the property of conferring upon living pneumococci the specific heritable characteristics of the strain from which it was derived. Among the characters which have been transferred in this way are serological type specificity, drug resistance, and the ability to synthesize specific enzymes (27). These transformations are permanent. The transformed cells multiply indefinitely as of the new type, and they produce transforming agents of the same kind as were used to transform them. The transforming agents are thus self-duplicating. That they are also mutable is indicated by the parallelism which exists between mutant strains of pneumococcus and the transforming principles which they produce.

Chemically, the transforming principle consists of DNA (28). Attempts to demonstrate the presence of proteins in purified preparations have not succeeded. The same thing is true of the transforming agents which have more recently been found in the unrelated bacterial species, *Hemophilis influenzae* (29).

Besides these two bacterial species, chemical identification has been made of the genetic material of two viruses—coliphage T2 and tobacco mosaic virus (TMV). Other speakers on this symposium will discuss the elegant chemical investigations which have been carried out on these forms. I will merely state the genetically interesting conclusion—which is that of the two major components that make up these viruses, protein and nucleic acid, the nucleic acid is the genetically active part—DNA in the bacteriophage, RNA in the tobacco mosaic virus (30, 31).

All the available evidence thus points toward nucleic acid, not nucleoprotein, as the ultimate genetic material. Much interest therefore attaches to the structure proposed three years ago for DNA by Watson and Crick (32). This structure not only accounts for the crystallographic and analytical data on which it was based, but it also seems capable of accounting for the genic properties of self-replication, mutation, and specific biological action. In fact, the self-duplication and mutation features follow so naturally from the proposed structure that they would have been predicted as properties of the molecule even without prior knowledge of the genetic role of DNA. Because of this, it will be surprising if the complementary two-stranded aspect, which gives the model its peculiar biological interest (33), is not supported by future investigations. With respect to the third genic property—specific biological activity—we are on less certain ground. The specificity of the Watson and Crick structure rests on the sequence of nucleotides, suggesting that the genetic information is coded on a linear tape in an alphabet of four symbols. The difficulty is that no mechanism is known at the present time for translating instructions from this alphabet into the alphabet of twenty-odd symbols which characterizes the proteins. The adequacy of this model as a description of the

gene rests to a large degree on the question of whether future work will disclose such a mechanism. In the meantime, the model provides for the first time a definite working hypothesis of gene structure which is already influencing the interpretation of genetic experiments.

There is, however, a puzzling feature about the present situation. The experiments of Fraenkel-Conrat (31) on tobacco mosaic virus clearly show that RNA is capable of performing a genetic function. Now it is well known that pentose nucleic acids occur in the cytoplasm of plant and animal cells, especially in the microsomes, and one might therefore expect to find a genetics of RNA in the cytoplasm comparable to that of DNA in the nucleus. This is precisely what is not found. Genetic experimentation with animals has consistently failed to reveal an extra-chromosomal hereditary mechanism other than that which is occasionally introduced into the cell by virus infection. In plants, a system of cytoplasmic inheritance does exist, but this system is of minor significance compared to the chromosomal mechanism. Unless there is a systematic error of some kind which prevents the detection of cytoplasmic inheritance in genetic experiments, these results imply the existence of two kinds of RNA. One kind, found in the plant viruses, is self-duplicating and mutable; the other kind, found generally in the cytoplasm, is genetically inactive. I know of no chemical distinction between RNA's from these different sources, but conceivably one exists. It is possible, for example, that the genetic form of RNA is a straight-chain molecule, like DNA, whereas the non-genetic form is branched.

By way of conclusion, one can say that real progress has been made in the chemical interpretation of genetic phenomena—much more than most of us thought possible even 15 years ago. The solution of one problem always raises another, however, and I have indicated some of the problems which are now bothering biochemical geneticists. What the solution of these problems will be, I do not know, but of one thing I am fairly certain: when the next semi-centennial of our Society comes around they will have ceased to trouble us.

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CHEMICAL PATHWAYS OF NUCLEIC ACID BIOSYNTHESIS[1]

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THE HISTORY of definitive information on pathways of nucleic acid biosynthesis covers barely a decade. Although 80 years ago Miescher (1) recognized that his 'nucleinstoffe' could arise from other cellular components, information on specific metabolic pathways leading to the polynucleotides began to be obtained only with the advent of isotope techniques. Progress has been so rapid since then that even an outline of the last ten years' accumulation of facts is not feasible, but I would like to mention a couple of the developments in the area of purine metabolism which are now in the process of evolution. Schoenheimer, with Barnes (2), initially studied the incorporation of isotopic NH_3, and from the laboratories of Buchanan (3) and of Greenberg (4), we have, in recent years, acquired a good picture of the manner by which purine derivatives are assembled from small precursors. A fairly complete picture of the origin of pyrimidine derivatives is also emerging. The largest single unit entering into the biosynthesis of a purine is glycine. This is coupled with an aminoribosephosphate at an early stage, and the remaining atoms necessary to complete the purine ring are then added. Thus the first complete purine derivative which results is a purine nucleotide. There is no evidence of the direct formation of any free purines. However, despite the fact that they are not intermediates on the pathways of biosynthesis of nucleotides, mechanisms do exist for the incorporation of exogenously supplied bases. Schoenheimer, with Plentl (5), had first investigated this possibility, but they had the misfortune to select three bases which were not utilized by the rat to an appreciable degree, and, unfortunately, drew the broad conclusion that administered purines and pyrimidines could not be utilized for anabolic purposes. Three years later it was found in our laboratory (6) that adenine was readily utilized by the rat.

Figure 1 is a very simplified outline of our present conception of the pathways leading to purines of nucleic acids. The first purine derivative which arises in the synthesis *de novo*, the 'common intermediate', is a purine nucleotide. We also know that administered purines are initially converted into nucleotide derivatives, represented by 'active adenine' and 'active guanine' in the figure. This scheme shows that adenine derivatives can be converted into guanine derivatives and vice versa. It indicates, too, that guanine can be utilized, which is true for many species. The facility with which either purine can be utilized and converted into the other varies greatly in different species. Recently a guanine-less mutant of *Aerobacter aerogenes* (7) was shown to be blocked in the synthesis *de novo* of the 'active guanine' derivatives, but it was still able to convert exogenously supplied guanine into polynucleotide adenine (8). This furnished a direct demonstration of the long-suspected existence of some pathway for the interconversion of purines which involves something more than a simple reversal of reactions leading from the common intermediate. That pathway is implied by the vertical broken arrows. This interconversion of adenine and guanine derivatives involves the appearance of a functional group in lieu of a hydrogen on position-2 and vice versa (fig. 2).

We have recently been interested in the chemistry and biology of purine, the unsubstituted parent ring. A riboside of purine (9-β-*d*-ribofuranosylpurine (9), Nebularin (10)) has been found in a mushroom (10), and studies of the metabolism of this riboside by M. P. Gordon (11) have shown that, in the rat, this compound can also be transformed into polynucleotide adenine and guanine. That transformation is the only example of the appearance of a functional group in lieu of a hydrogen on the position-6 of a

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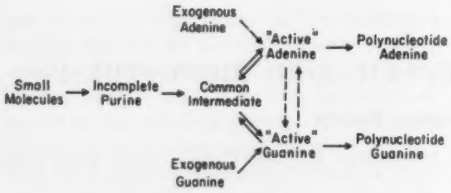

FIG. 1. Metabolic pathways of purine derivatives arising from synthesis *de novo*, of exogenous purines, and of the interconversions of the purines.

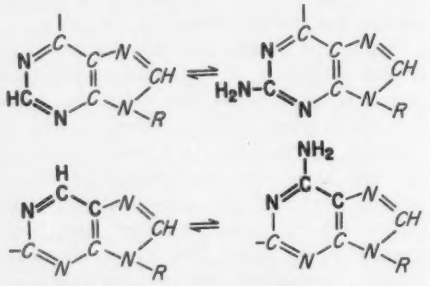

FIG. 2. Transformations occurring at the 2- and 6-positions of purines.

purine ring. Unless one is to postulate a totally independent origin in the mushroom, it is to be assumed that its biosynthesis from one of the more usual purine derivatives must involve the replacement of a functional group in position-6 by a hydrogen. The details of the mechanisms of the introduction and removal of such substituents are only beginning to be revealed. Xanthylic acid appears to be an intermediate in the conversion of inosinic acid to guanylic acid (12–14), and a further development concerning the reversal of that transformation, one which appears to involve a removal of the 2-carbon, is to be reported by Drs. Moyed and Magasanik (15). The problem of a mechanism for the analogous replacement which can occur at the 6-position (fig. 2) remains obscure.

In addition to the utilizations of the purine bases, base-labeled samples of many of the nucleosides and nucleotides which can be derived from the nucleic acids have also been studied in the rat in the last five years. Nucleosides and nucleotides of the pyrimidines, cytosine and uracil, and of the nucleotide of guanine, proved to be utilized far more readily than were the corresponding free bases.

In order to ascertain something of the mechanisms by which the various units of the nucleotide molecules were utilized, Drs Roll and Weinfeld (16–19) carried out studies with several multiple-labeled (N^{15}, C^{14} and P^{32}) nucleotides, and followed the fate of each major segment of the molecules. These were the 2′-and 3′-nucleoside phosphates, which are those obtained by alkaline degradation of suitably labeled RNA. There was no specific incorporation of the phosphates (P^{32}), and subsequent studies (20) have demonstrated that injected nucleoside 2′- or 3′-phosphates lead to the 5′-phosphates of the soluble nucleotide fraction; this must involve incorporation of other phosphate which has been in equilibrium with the inorganic phosphate. The most informative results were obtained by isolating the individual nucleotides from the nucleic acids, and degrading these to permit separate measurements of the appearance of the isotopes N^{15} and C^{14} in the bases and C^{14} in the riboses from each nucleotide.

After the administration of the cytosine nucleotide, the ratio of the isotopic label in the RNA cytosine and in its accompanying ribose was the same as that in the administered cytidylic acid. Thus, the cytosine-ribose unit was the one which was utilized intact. It was also converted into uracil derivatives without cleavage of the glycosyl linkage. The deoxyribosyl derivatives likewise showed a ratio of isotope in the base and deoxyribose which suggests and extensive origin of deoxyribose derivatives by way of a reductive removal of oxygen from the second carbon, a reaction which has not been suggested by any of the studies with isolated enzyme preparations. The behavior of the uridine moiety of uridylic acid was similar, but with isotope values which were all much lower because of the considerably less efficient utilization of the uracil derivative. These results fully confirm and extend the suggestions made by Hammarsten, Reichard and Saluste (21) on the basis of the fates of pyrimidine ribosides and deoxyribosides, and those of Rose and Schweigert (22) with uniformly labeled cytidine.

Similar studies with purine ribotides led to a quite different picture. With them, there was extensive incorporation of the ribose, but the ratios did not approach 1.0. The values for the sugars were high enough, and there was a sufficiently specific incorporation into the purine nucleotides to indicate that this was not a nonspecific utilization of small fragments. However, it was obvious that some purines reached the

polynucleotides without being accompanied by their original ribose. This was most evident with the RNA guanylic acids, since a maximum of about 20% of the guanine finally incorporated could have been attached to its original ribose.

In view of several differences in the behaviors of free purines and their ribosyl derivatives, particularly of guanine and guanylic acid in the rat, it seems that the appearance of the purine attached to a new glycosyl radical may well be the result of a type of transglycosidation reaction. We have referred (23, 24) to this process as 'transpurination' to stress that part of the molecule, and to emphasize the fact that, in the rat, this is observed with purine derivatives but not with pyrimidine derivatives.

The evidence demonstrates that there is some transfer of purines to new ribosyl derivatives at some unknown stage in the metabolic pathways leading to the polynucleotides. The postulation that such a 'transpurination' can occur between some nucleotide and a pre-existing polynucleotide offers advantages in reconciling several of the observations on both RNA and DNA which have indicated a disproportionately large incorporation of preformed purine relative to that of small precursors. A transfer of purines from some ribosyl derivative directly onto the ribose-phosphate backbone of a nucleic acid is a mechanism which could account for an additional incorporation of a purine, independent of that involved in the total synthesis of polynucleotides from other precursors.[2]

Recent observations on the influence of x-irradiation on DNA synthesis may be interpreted as fully in accord with such a hypothesis. Harrington and Lavik (28) observed, with rat

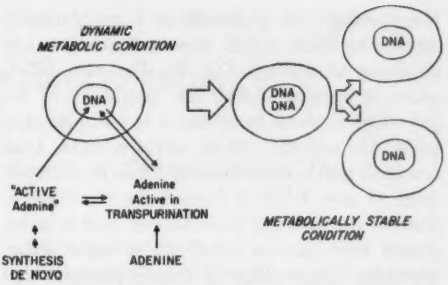

FIG. 3. Possible relationships of the synthesis of, and 'transpurination' with, DNA during an interphase period, and of its metabolic stability thereafter.

thymus, that irradiation could decrease the incorporation of formate, orotic acid and phosphate into DNA, but that incorporation of exogenously supplied adenine was not decreased. With the chick embryo, Passoneau and Totter (29) have similarly observed that irradiation can reduce incorporations of formate and glycine without depressing that of adenine. An interference with the total synthesis of DNA, but one which does not interfere with the transpurinatoin of the exogenously supplied purine into the already existing DNA, is a possible explanation of those observations on metabolic behaviors following irradiation.

The mass of available evidence does indicate that DNA is in a highly active metabolic state during that portion of interphase when new DNA for daughter cells is being formed (fig. 3), and we now postulate that transpurination as well as total synthesis may occur in that active metabolic state. However, in tissues which have ceased rapid growth, the DNA shows little metabolic activity. A long-term retention of the isotopes incorporated into the purines during the formative phase, regardless of the precursor which was used to introduce them, suggests that the DNA in a non-dividing cell is in a stable condition in which neither total synthesis and degradation nor transpurination takes place.

The lines of evidence which indicate multiple mechanisms during the synthesis of DNA and those indicating prolonged and uniform retention of the DNA once it is formed can be reconciled[3] if it be assumed that the bulk of the DNA in

[2] Introduction of pyrimidines into the polynucleotides of certain microorganisms may occur by a similar transglycosidation mechanism. In contrast with the rat many microorganisms utilize pyrimidines: an enzymatic transglycosidation of pyrimidines by bacterial enzymes has been demonstrated (25), and *Escherichia coli* cleaves cytidine in the course of its utilization (22). Weygand *et al.* (26) and Zamenhoff *et al.* (27) have observed the incorporation of bromouracil into the DNA of *E. coli* under conditions which suggest that incorporation of the pyrimidine into DNA can be independent of total synthesis, and Dr. Herbert Pahl has obtained evidence suggesting the same thing (unpublished). In the rat the non-utilization of pyrimidines and the fact that pyrimidine nucleoside units are used intact suggests that there is a pronounced difference between the rat and certain microorganisms.

[3] Some of the implications of transpurination with respect to existing data on the synthesis of DNA from various precursors have been discussed in greater detail (24).

non-dividing cells is present in a metabolically stable condition which does not involve any synthesis or renewal (fig. 3). The new DNA, which is forming during the interphase of the cell which is about to divide, is in a condition of metabolic activity which includes both total synthesis and transpurination. Once the complement of new DNA is formed, its condition or state of combination is so altered that it is excluded from further metabolic changes: either synthesis, degradation or transpurination, and the daughter cells remain in that condition until they again prepare to divide.

These instances where isotope techniques have revealed the pronounced differences between the behaviors of the nucleoside portions of pyrimidine and purine nucleotides, and the introductions of new groups, at the 6- as well as at the 2-positions of purines, pose new questions. More detailed mechanisms, and enzymes catalyzing those transformations, will no doubt be elucidated before long. I would hesitate to predict what our over-all 'flow sheet' of the chemical pathways of nucleic acid biosynthesis will be in the future, even one week in advance when that week includes the ASBC meetings. Applications of the recognized precursors to studies of physiological processes are certainly adding to our knowledge of the processes of growth, multiplication, protein synthesis and virus propagation. Perhaps knowledge of these pathways will permit applications of the antimetabolite concept which can lead to effective controls of neoplastic growth, viral infections or other metabolic aberrations, areas where medicine and biochemistry blend.

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BACTERIOPHAGE AS NUCLEOPROTEIN[1]

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OUR BIOCHEMICAL INFORMATION concerning the bacterial viruses is derived primarily from the so-called T series of coliphages, active on *E. coli*, strain B. Since knowledge of some members of this series is quite limited, I have preferred to confine the discussion to a single strain of bacterial virus, namely, coliphage T_2. My intention is to outline the contemporary information concerning the various chemical components of the virus particle and then to proceed to a discussion of the extent to which these can be correlated with our knowledge of the mechanism of viral replication.

To refer to the phage particles as nucleoproteins (as I have done in my title) is simply to emphasize the fact that these agents are largely composed of nucleic acid and protein, that both components are essential to their physiological activity, although all of the available evidence suggests that these two components have quite separate and distinct physiological functions.

Figure 1 is a diagrammatic representation of the T_2 particle indicating current views as to function. The particles have a slightly elongated hexagonal head attached to a smaller tail, terminating in a thickened end-structure of the indicated dimensions. Highly purified preparations of the virus can be obtained which exhibit homogeneity in both the ultracentrifuge and the electrophoretic apparatus. From a variety of analytical procedures, both physica and biological, it would appear that the particle weight is of the order of 200,000,000. Chemical analysis indicates that about 50% of the nitrogen is present as nucleic acid of the DNA type with the rest being protein. Most virus preparations contain measurable quantities of lipid material (about 2%) but it is uncertain whether this is a contaminant or an integral part of the viral structure.

Such viral particles as T_2 do not show demonstrable metabolic activity, do not contain energy 'reservoirs', nor require any source of energy to maintain their structure. It appears, therefore, that we are dealing with particles which although enormous in molecular size are bound together by the usual covalent bonds as well as by such other types of intra-molecular binding forces as operate, for example, in determining the particular configurations of a biologically active protein molecule.

Although it is not yet possible to separate and recombine the nucleic acid and protein components of phage in a manner similar to that which has been so elegantly demonstrated for tobacco mosaic virus, a number of physical and chemical procedures now exist which indicate heterogeneity in both the protein and nucleic acid components.

As indicated in the diagram (fig. 1), it is generally believed that the nucleic acid of the virus particle is concentrated mainly within the area of the hexagonal head and is sheathed in a protective coat of protein. The view is based on the fact that the intact virus particle is immune to the action of DNAase, and more particularly to the observation that if one rapidly dilutes suspensions of T_2 in concentrated salt solutions, the nucleic acid is liberated into the medium and the remaining protein 'ghosts' are seen, under the electron microscope, to retain the sperm-like shape of the intact particle, although the hexagonal head is empty. The DNA released by this procedure can be hydrolyzed by DNAase and is probably highly disorganized as compared to the state in which it existed in the intact particle.

If the genetic material of the phage is uniquely DNA, then the DNA structure must be such as to permit the existence of some thousands of areas of specific chemical dissimilarity in order to explain the existence of numerous genetic qualities. However, such areas can be present as united components of a single thread-like fibrous structure. Recently Brown and Martin (1) have been able to separate the DNA from

[1] The original experiments included in this report were supported by grants from the National Foundation for Infantile Paralysis, Inc., and from the Dr. Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago.

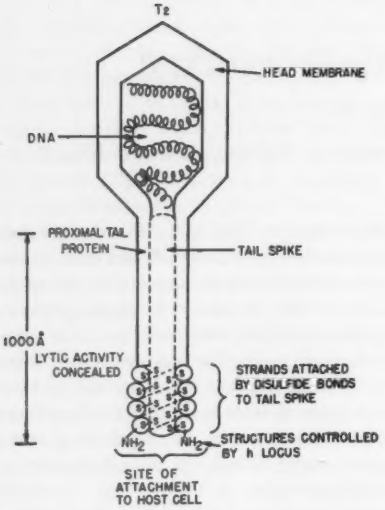

FIG. 1

T_2 into two fractions by use of a column of cellulose and calf-thymus histone. The two portions, 30 and 70% respectively of the total nucleic acid, are characterized by differing ratios of purine and pyrimidine bases. Further evidence for the heterogeneity of viral DNA is derived from recent experiments of Levinthal (2) which I shall discuss somewhat later.

The most striking feature of the chemistry of the DNA of the T_2 particle is the presence of several anomalous components. Wyatt and Cohen (3) discovered that the even-numbered T phages contained a new pyrimidine, 5-hydroxy methyl cytosine, which is absent from the nucleic acid of the host cell and appears to replace cytosine entirely in the DNA of this group of phages. Sinsheimer (4) and, independently, Volkin (5) demonstrated that glucose is also an integral component of T_2 phage, occurring probably in glycosidic combination with the 5-hydroxy methyl group. One is inclined to regard these variations as being of great, if unknown, physiological significance in the function of T_2 but since comparable anomalies have not as yet been found in either the odd-numbered T phages or the temperate phage λ, it has not yet been proved that they constitute a general pattern in viral composition.

Evidence is also accumulating with respect to the heterogeneity of the protein component of T_2 phage. Lanni and Lanni (6) in 1953 showed the presence of at least two distinct antigens in viral preparations, one associated with the protein of the head and the other with the protein of the tail. At the moment, we have evidence for at least four distinguishable protein fractions. Our most recent information in this respect has been derived largely from the work of Kozloff in our laboratory, of Kellenberger in Geneva, and of others. The four protein components are: *1*) the protein sheath of the hexagonal head; *2*) that of the proximal portion of the tail; *3*) that in the distal or terminal tail fragment; and *4*) that of an internal 'spike' or plug-like structure which is only revealed after appropriate treatment of the intact virus particle although the evidence for protein nature here is still insufficient.

Kozloff and Henderson (7) have shown that by treatment of T_2 with divalent Zn^{++} or Cd^{++} in the presence of a complexing agent, the distal portion of the tail is split off, inactivating the phage. A number of complexing agents are active, such as various amino acids and proteins, although cyanide has proved to be most convenient for much of the experimental work. Neither the metallic ion nor the complexing agent is effective alone—both are required for the liberation of the distal portion of the tail. In preparations of T_2 treated with Cd^{++} and cyanide, one finds that all of the particles retain the intact head structure as well as the proximal portion of the tail, although this has thickened somewhat, possibly due to retraction of the protein coat. In such preparations the electron microscope reveals the presence of a smaller number of phage particles (10–20%) in which the removal of the distal portion of the tail and the thickening of the proximal part has exposed a terminal 'spike'. Such preparations also contain a number of particles having the appearance and dimension of the spike and one can see similar structures in lysates of infected bacterial cells and in lysates from infected cells in which the normal replication of the virus particle has been prevented by proflavin. As shown in the diagram (fig. 2) it seems very probable that the phage particles contain an internal spike that can be seen only when the distal portion of the tail is removed and which, under such circumstances, can be readily removed from the tail. Similar conclusions arise from the independent investigations of Kellenberger and his co-workers (8) on the oxidation of T_2 by 3% H_2O_2 in 10% ethyl alcohol. The Swiss workers describe first the appear-

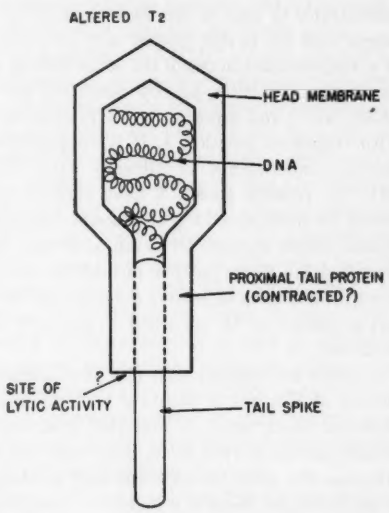

FIG. 2

ance of thread-like structures (varying from three to five in number) unravelling from the very tip of the tail. If oxidation proceeds long enough, the distal portion of the tail is entirely removed and the thickening or retraction of the proximal portion of the tail clearly reveals a spike identical in appearance with that obtained by treatment with cadmium cyanide.

I have not time here to describe the matter in detail, but Dr. Kozloff will present evidence at another session of this meeting (9) that the effect of Cd^{++} or Zn^{++} complexes is to rupture disulfide bonds present in the protein sheath of the tail structure so that the distal portion of the tail is liberated. The mechanism is probably one of hydrolysis, but a similar effect can be exerted by thioglycollic acid, presumably reducing the same disulfide linkages to the sulfhydryl state. The effect of Cd cyanide is quite specific and both the head structure and the proximal tail portion of the particle are undamaged, although, as I shall describe later, the DNA inside the head may be removed by appropriate treatment. However, with alcoholic H_2O_2, Kellenberger has found that prolonged treatment of T_2 results eventually in damage to the head structure, liberating DNA, although the proximal portion of the tail seems unchanged. Kozloff has also observed differential susceptibility or sensitivity of the head protein, inasmuch as exposure of the Cd-cyanide treated particle to 0.2 M arginine leads to a rapid destruction of the head structure, although the proximal portion of the tail remains intact. Kellenberger has studied the effect of DNAase on the spikes observed in a number of preparations, and finds them unaltered (8), so that it seems probable in terms of the composition of the intact particle, that they are protein in nature although other chemical components cannot be excluded.

These facts represent a summary of our contemporary information in regard to the various protein and nucleic acid components of the phage particle, and I should like to proceed to a discussion of our information regarding the role of these various materials in the process of virus replication.

Anderson (10) first showed that the T_2 particle is adsorbed to the specific bacterial host by the end structure of the tail. Garen and Puck (11) demonstrated that the initial phase of the absorption process is reversible, and depends on the existence of complementary electrostatic and geometric relationships between the virus and receptor areas of the host cell wall. Puck and Tolmach (12) were able to show further that with T_2 the initial interaction involves positively charged amino groups—presumably on the protein of the distal portion of the tail—with negatively charged carboxyl groups present on the bacterial cell surface. The first reversible process leads to an irreversible phase which probably involves changes in both the virus particle and the bacterial cell wall. Under normal conditions, this irreversible phase of adsorption is followed by a splitting of the coliphage particle so that the bulk of the nucleic acid of the phage particle passes into the bacterial cell. The protein membrane remains attached to the exterior of the cell and, as shown by the elegant isotope experiments of Hershey and Chase (13), can be removed without interfering with the further stages of virus reproduction, simply by agitating the infected bacterial cells in a Waring blendor.

Further insight into the detailed sequence of events involved in the invasion of the particle has recently been obtained by Kozloff, Barrington and Brown (14, 15) in a study of the interaction between T_2 and so-called bacterial cell walls. These are prepared by autolysis of heavy suspensions of *E. coli* in buffer solutions and subsequent treatment with proteolytic enzymes and lysozyme. Such preparations are homogeneous and specifically adsorb and inactivate coliphages which attack the original bacterial cell. The process can be studied by using bacterial

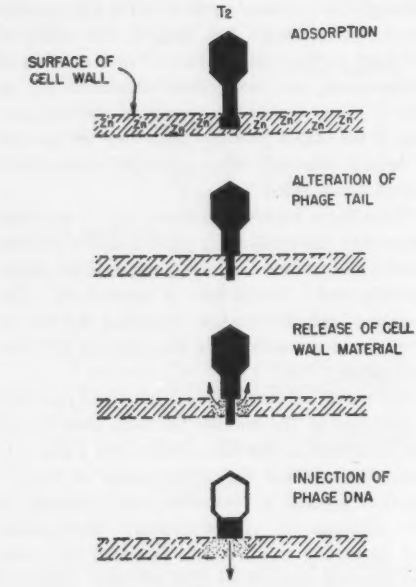

FIG. 3

membranes in which the nitrogen has been marked with isotopic N^{15} or C^{14}. When such membranes are treated with T_2, quantities of soluble compounds are released in direct proportionality to the quantity of phage added. If one studies this lytic process, comparing the effect of the three types of particles, i.e. intact T_2 particles, of particles without the distal portion of the tail but still retaining the spike, and of particles in which both the distal tail and spike have been removed, one finds the lytic activity of the latter species is more pronounced than that of the first two. It seems probable, therefore, that the initial interaction between the protein of the tip of the phage tail and the bacterial cell surface is followed by a reaction in which the distal portion of the tail is removed, so that lysis of the bacterial surface is occasioned by contact between the cell and the proximal portion of the phage tail.

The sequence of events would be those represented diagrammatically in figure 3 involving *1*) interaction between virus and host, *2*) splitting off of the distal portion of the tail, *3*) lysis of the adjacent areas of the bacterial cell wall, permitting *4*) expulsion of the internal spike, together with viral DNA into the bacterial cell proper. There are several comments that can be made with reference to this sequence. In the case of *step 2*, it is possible to demonstrate sufficient concentrates of zinc in the bacterial cell wall to believe that the *in vivo* process may be catalyzed by a zinc complex in much the same fashion and the *in vitro* reaction, i.e. by fusion of the disulfide bonds and exposure of the internal spike. With regard to reaction 4, it is appropriate to mention experiments of Hershey (16). Along with the protein ghosts I have already mentioned, he finds some 3% of a non-sedimentable protein which appears to be an authentic component of the phage particle but differs from the ghost protein both in lacking antigenic properties and in failure to be adsorbed to the host bacterial cell.

It seems not unlikely that this unsedimentable protein of Hershey is identical with the spiked structure which must be extruded from the attached particle if viral DNA is to enter the cell. Whether the spike as such has any mechanical or physiological effect is a matter of speculation. It would be of interest to ascertain whether it has any enzymic activity: for example, in attacking the DNAase inhibitor present in the bacterial cell which disappears after infection, with a consequent elevation of the DNAase activity of the infected cell.

The nature of the forces responsible for the injection of viral nucleic acid into the bacterial cell is entirely obscure. With particles in which the distal portion of the tail has been removed with cadmium cyanide, one observes a partial emptying out of DNA into the medium. This can be accelerated by a variety of compounds: amino acids, amines, amino sugars, with the active compound in every case possessing a positively charged amino nitrogen (15).

It is not possible to detect the presence of intact virus during the next phase of viral reproduction. If the cells are disrupted during this period, one finds particles which are protein in nature, related serologically to virus protein, but incapable of inducing the virus infection and representing probably intermediate states in viral synthesis. Since the DNA of T_2 contains 5-HMC and is free of cytosine, one can study the synthesis of the nucleic acid portion of the virus by following the changes in phage specific DNA and of host specific DNA. It is found that phage DNA increases some 7 min. after infection while the bacterial DNA decreases. At a time approximately half the period between infection and lysis, disruption of the cell shows that mature infectious virus particles are beginning to appear. However, viral DNA continues to

increase and remains in excess of that present as infected particle until the cell finally lyses.

The synthesis of 5-HMC by the infected cell would seem to require the preliminary formation of enzymes necessary for its production since the pyrimidine does not occur in the bacterial cell. Burton (17), by the use of amino acid-requiring mutants of *E. coli*, has shown that after infection of the host cell there is an obligatory synthesis of protein before the synthesis of the characteristic 5-HMC-containing phage is initiated. Once this obligatory protein has been synthesized, the subsequent synthesis of phage is largely independent of the synthesis of protein containing tyrosine or tryptophan, although protein must be formed for the protective coat of the particle. Similar conclusions using chloramphenicol as an inhibitor of protein synthesis have been reported by Timizawa and Sunakawa (18).

The mature virus particle may contain material derived from the parent infecting particle, the bacterial host cell and the nutrient components of the medium. Since the bulk of the protein membrane of the virus particle remains outside the infecting cell, it is clear that it cannot contribute to the protein of the viral progeny and isotopic experiments support this conclusion. The question of the direct utilization of parental DNA on the DNA of the progeny has been a matter of considerable investigation and conjecture. Studies on the fate of labeled viral DNA have shown that immediately after its injection into the host cell a considerable portion can be detected in the acid-soluble material fraction so that some further subdivision of the injected material must be taking place. A number of isotopic tracer studies by various workers led to the general conclusion that there was no possible correlation between the material of DNA from the infecting phage and the transfer of hereditary units although parental DNA must be the agent whereby these units are transmitted. And indeed, one can observe the transfer of considerable quantities of isotope from labeled parents to progeny under circumstances in which genetic transfer seems excluded. Levinthal (2) has recently developed techniques which allow him to measure the radioactivity of a single virus particle labelled with P^{32} if its atoms emit more than 10 beta particles per month. This involves surrounding the particle under study with a photographic emulsion sufficiently sensitive so that fast electrons leave a visible track which can be observed directly under the microscope. Levinthal's results indicate that the nucleic acid of T_2 consists of one large piece and a number of smaller pieces. The small pieces of DNA are distributed among a large number of progeny virus while the large piece of DNA is transmitted in an intact state to one of the viral progeny. A copy of this essential nuclear core must then be synthesized *de novo* for the remaining virus particles synthesized in the infected cell. A final decision as to this matter is clearly of the greatest importance in our understanding of the mechanism of viral replication.

Perhaps the most striking feature of the utilization of the components of the host cell for virus synthesis is the use of practically all of the bacterial DNA. This first involves breakdown of the bacterial nucleic acid into smaller fragments which are then used for the synthesis of viral DNA and especially for the viral particles which are formed in the early stages of the infectious process. To the extent that there is sufficient bacterial DNA to account for the whole of the DNA of the viral progeny, relatively little synthesis of viral DNA from the components of the medium takes place. However, as is the case with T_2, when bacterial DNA is insufficient in amount for the DNA of the viral progeny, and when there is necessity for the synthesis of a new component, such as 5-HMC (synthesized in part from the cytosine of the host) then this additional material is synthesized from the simple components of the medium. There is little or no utilization of bacterial protein or amino acids for virus synthesis, and viral protein is formed largely by *de novo* synthesis from the components of the medium.

The picture of virulent coliphage infection which emerges from this is one in which the process of virus synthesis is set off by a fragment of the original infecting particle. The machinery that is used for virus synthesis is for the most part the normal metabolic equipment of the bacterial cell, and the materials on which it operates are the normal components of the bacterial environment and certain portions of the bacterial cellular fabric. Many, if not all, of the normal metabolic functions of the bacterial cell cease, and there begins an immediate and rapid synthesis of the specific parts of the virus particle. Viral DNA appears rapidly. Viral protein can be detected somewhat later, but apparently the two components of the mature particle are assembled only

at a terminal stage of the replication process. Beyond this our biochemical information proceeds for only a short distance.

If one asks what is the mechanism by which this alteration of the normal metabolic activities of the growing cell is effected by a fragment of viral DNA, one can only point to the obscurity of our knowledge in respect to the metabolism and synthesis of nucleic acid and of protein. Both cytological and chemical evidence point to the fact that the replication of T_2 virus involves a gross disturbance in the normal metabolism of the nuclear material of the sensitive bacterial cell. Finally, I might add that such a view is also reinforced by what we know of the behavior of the so-called temperate viruses, a subject which I cannot discuss in further detail here.

Many of the phenomena of virus infection and duplication have hitherto been more amenable to the dexterous explorations of the microbiologist and the geneticist than to those of the chemist. We are just at the beginning of the attempt to translate such phenomena into chemical language, although some small progress has been made in carrying the description to the ultimate molecular level.

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ENZYMATIC SYNTHESIS OF RIBONUCLEIC ACID-LIKE POLYNUCLEOTIDES[1]

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The mechanisms of synthesis of the polynucleotide chains of nucleic acids have remained obscure despite notable advances in our understanding of the enzymatic mechanisms involved in the synthesis of the mononucleotides, the purine and pyrimidine bases, and the sugar moieties.

As reported in several publications from our laboratory (1–3), an enzyme isolated, and partially purified, from the micro-organism *Azotobacter vinelandii* catalyzes the synthesis of highly polymerized ribopolynucleotides from 5′-nucleoside-diphosphates with release of orthophosphate. The reaction requires magnesium ions and is reversible. The available evidence indicates that the *Azotobacter* enzyme catalyzes the reaction

$$n\text{X} - \text{R} - \text{P} - \text{P} \rightleftharpoons (\text{X} - \text{R} - \text{P})_n + n\text{P}$$

where R stands for ribose, P-P for pyrophosphate, P for orthophosphate and X for one or more of the following bases: adenine, hypoxanthine, quanine, uracil or cytosine.

Chemical and enzymatic degradation of the

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biosynthetic polynucleotides, carried out in collaboration with Dr. Leon A. Heppel of the National Institutes of Health, showed that they are made up of 5′-mononucleotide units linked to one another through 5′,3′-phosphoribose diester bonds as in RNA[2] (cf. fig. 2). Thus, in analogy with polysaccharides, reversible phosphorolysis may be a major mechanism in the biological synthesis and breakdown of polynucleotide chains. For this reason, the name polynucleotide phosphorylase was proposed (1) for the new enzyme. Evidence has been obtained that this enzyme brings about the synthesis of RNA-like polynucleotides. It has been found in our laboratory (4) that polynucleotide phosphorylase is widely distributed in bacteria and the presence of the enzyme in some microorganisms has been confirmed by other investigators.

Single polymers containing AMP, GMP, UMP, CMP, or IMP as the only basic unit have been obtained by incubating polynucleotide phosphorylase with the corresponding 5′-nucleoside diphosphates. They are referred to as the AMP(A), GMP(G), UMP(U), CMP(C), or IMP(I) polymers, respectively. To date, two 'mixed' polymers have been prepared, one from approximately equimolar mixtures of ADP and UDP and one from mixtures of ADP, GDP, UDP, and CDP, in approximately molar proportion 1:0.5:1:1. These polymers are referred to as the AU, and the AGUC polymers, respectively.

The possibility that the 5′-nucleoside diphosphates could be the direct precursors of RNA has been considered by Potter (5). The 5′-mono-, di-, and triphosphates of guanosine, uridine and cytidine have recently been shown to be present in animal tissues (6). The mechanism of formation of the 5′-polyphosphates has been elucidated through the discovery (7) of nucleoside monophosphate kinases catalyzing the transfer of phosphate from ATP to GMP, UMP, or CMP, and from ADP to GDP, UDP, CDP, or IDP.

On incubation of polynucleotide phosphorylase with nucleoside diphosphates in the presence of Mg^{++}, there is a disappearance of nucleoside diphosphate with liberation of a stoichiometric amount of orthophosphate. The reaction reaches equilibrium and comes to a standstill when about 60 to 80% of the nucleoside diphosphate has disappeared. The time course of orthophosphate liberation is shown in figure 1 for the various single and 'mixed' polynucleotides so far prepared. The polynucleotides are isolated by precipitation with cold ethanol and purified by solution in a small volume of water, reprecipitation with ethanol and extensive dialysis against distilled water. They are recovered as white powders by lyophilization of the aqueous solutions.

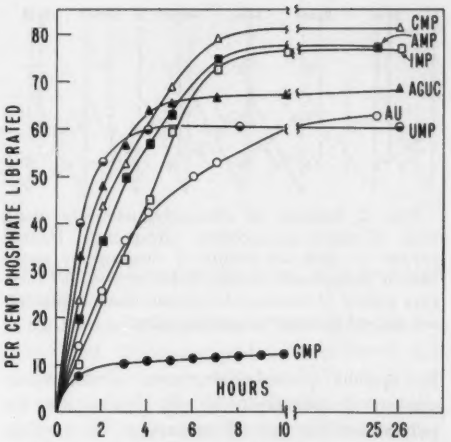

FIG. 1. Orthophosphate liberation during synthesis of polymers (3). All samples contained tris (hydroxymethyl)aminomethane-HCl buffer, pH 8.1, $MgCl_2$, and partially purified *Azotobacter* polynucleotide phosphorylase. For single polymers 50 mg/ml were used of each of the following nucleoside diphosphates, ADP, IDP, UDP and CDP, and 25 mg/ml of GDP. The sample for AU polymer contained 25 mg each of ADP and UDP per ml; that for AGUC polymer contained 7 mg/ml each of ADP, UDP and CDP, and 3.5 mg/ml of GDP. Incubation at 30°. For further details see ref. 3. The ordinate gives the percentage of acid-labile phosphate released as orthophosphate.

STRUCTURE OF POLYNUCLEOTIDES

The polynucleotides synthesized by polynucleotide phosphorylase consist (2, 8) of 5′-nucleoside monophosphate units linked to one another through 5′-, 3′-phosphoribose diester bonds. The chains are terminated by phosphate groups sterified at carbon 5′ of the nucleoside moiety. This is shown diagrammatically in figure 2 which also indicates the points at which the chain is cleaved

[2] The following abbreviations are used: RNA, ribonucleic acid; DNA, deoxyribonucleic acid; ADP, GDP, UDP, CDP, and IDP, 5′-diphosphates (pyrophosphates) of adenosine, guanosine, uridine, cytidine, and inosine, respectively; AMP, GMP, UMP, CMP, and IMP, 5′-monophosphates of the same nucleosides; ATP, adenosine-5′-triphosphate.

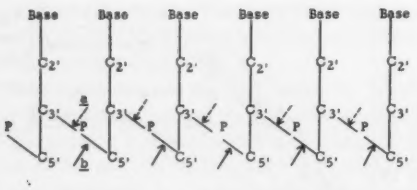

FIG. 2. Scheme of ribo-polynucleotide chain with 5′-phosphomonoester end-group. Dotted arrows (*a*) indicate points of cleavage by snake venom phosphodiesterase. Solid arrows (*b*) indicate points of cleavage by spleen phosphodiesterase and of primary attack by alkali.

by specific phosphodiesterases. Thus, snake venom phosphodiesterase (9) hydrolyzes the polynucleotides to 5′-nucleoside monophosphates while spleen phosphodiesterase (10) yields the corresponding 3′-nucleoside monophosphates. The same products are obtained by hydrolysis of natural RNA with the diesterases (11, 12). Further, as is the case with natural RNA, mild alkaline hydrolysis of the biosynthetic polynucleotides liberates approximately equimolar mixtures of the 2′- and 3′-nucleoside monophosphates. Hydrolysis by alkali cleaves polynucleotide chains at the same points attacked by spleen phosphodiesterase (cf. fig. 2) but, as shown by Brown and Todd (12), alkaline hydrolysis proceeds with the liberation of 2′-, 3′-nucleoside monophosphates (cyclic nucleotides) which are then cleaved to give mixtures of the 2′- and 3′-nucleoside monophosphates. This was confirmed by Markham and Smith (13).

End Groups. It may be seen in figure 2 that alkaline digestion of a polynucleotide chain such as that represented will yield nucleoside monophosphates except at the ends which will be split off as nucleoside and nucleoside diphosphate. The separation of the three kinds of fragments, nucleosides, nucleoside monophosphates, and nucleoside diphosphates, by paper electrophoresis at neutral pH was developed by Markham, Matthews and Smith (14) as an end-group assay method which permitted them to show that the nucleic acids of certain plant viruses, such as tobacco mosaic virus and potato virus X, conform to the structure shown in figure 2. That the polynucleotides synthesized by polynucleotide phosphorylase possess the same structure is indicated by end-group assays carried out by Dr. J. D. Smith in our laboratory (8). Figure 3 shows ultraviolet prints of filter paper strips

FIG. 3. End-group assay of biosynthetic polynucleotides (J. D. Smith). Ultraviolet prints of paper electrophoresis (pH 7.4) strips. 1—A polymer after treatment with prostatic phhospomonoesterase; 2—A polymer; 3—AU polymer; 4—AGUC polymer. Origin indicated by notches at top. Fast moving band (toward anode), nucleoside diphosphates; middle band, nucleotides; slow moving band, nucleosides.

after electrophoresis of alkaline digestion mixtures of the A, AU, and AGUC polymers. The three major bands in strips 2 to 4 are from top to bottom, nucleosides, nucleoside monophosphates, and nucleoside diphosphates. Strip 1 shows the electrophoresis pattern of the alkaline digestion mixture of the A polymer after removal of the 5′-esterified phosphate group at one end (cf. fig. 2) by prostatic monoesterase. The nucleoside diphosphate band is missing in this strip.

Ribonuclease Digestion. On digestion with pancreatic ribonuclease biosynthetic polynucleotides yield the same hydrolysis products as RNA (8). Pancreatic ribonuclease is a phosphodiesterase with a characteristic specificity; it cleaves pyrimidine nucleoside phosphodiester bonds distal to a 3′-linkage. Figure 4 illustrates the action of ribonuclease on the AU polymer. The enzyme releases 3′-uridylic acid and polynucleotides containing one uridylic acid residue and one or more adenylic acid residues. Markham and Smith (13) have shown that 2′-, 3′-pyrimidine nucleoside monophosphates (cyclic pyrimidine mononucleotides), are intermediates in the cleavage of RNA by ribonuclease and are further

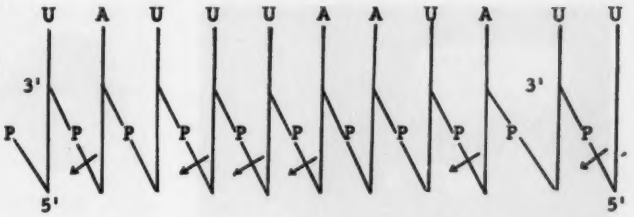

FIG. 4. Hypothetical chain of AU polymer and points of cleavage by pancreatic ribonuclease (arrows).

hydroylzed to the corresponding 3′-pyrimidine mononucleotides. Thus, cyclic pyrimidine mononucleotides can be obtained in larger or smaller amounts during digestion of RNA with ribonuclease. Dr. Heppel has been able to obtain large yields of cyclic uridylic acid by ribonuclease digestion of the U polymer.

On exhaustive ribonuclease digestion of the AU polymer, Heppel obtained 3′-uridylic acid along with a series of oligonucleotides, of which the mono-, di-, tri-, tetra-, and pentanucleotides have been characterized (8). The separation of these nucleotides by paper chromatography in ammonium acetate-ethanol (15) is shown in figure 5. The oligonucleotides consist in all cases of one uridylic acid residue with one or more adenylic acid residues and are terminated by a phosphomonoester group esterified at carbon 3′ of the uridine moiety. Hydrolysis of these oligonucleotides with semen phosphomonoesterase liberates the terminal phosphate group as orthophosphate and alkaline hydrolysis of the remaining compounds releases one mole of uridine and one or more moles of 2′- (3′-) adenylic acid.

When RNA is exhaustively digested with ribonuclease and the reaction mixture is dialyzed against distilled water there remains a non-dialyzable residue or 'core' which consists of a mixture of oligonucleotides with relatively high proportions of purine mononucleotides. Parallel hydrolyses of samples of the AGUC polymer and yeast RNA showed that in both cases there remained a non-dialyzable core amounting to 20–22% of the total nucleotides. Both Dr. Smith and Dr. Heppel (8) have carried out ribonuclease digestions of the AGUC polymer and have further resolved by electrophoresis, following the general technique of Markham and Smith (16), the oligonucleotide bands obtained from the digestion mixture by paper chromatography in the isopropanol-ammonia solvent system. This demonstrated the presence of guanylic-cytidylic, guanylic-uridylic, adenylic-cytidylic, and adenylic-uridylic dinucleotides along with trinucleotides (fig. 6).

Nucleotide Composition. Since there is evidence that ribonucleic acids from different cells or tissues vary in composition (17), it was of interest to compare the nucleotide composition of the AGUC polymer with that of RNA isolated from *Azotobacter vinelandii*. This has recently been done by Dr. R. M. S. Smellie in our laboratory (18) and his results are summarized in table 1. Upon hydrolysis of the polynucleotides with perchloric acid, the purine and pyrimidine bases were separated by paper chromatography in the

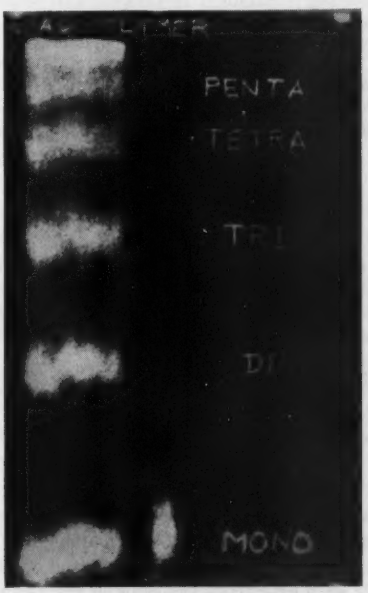

FIG. 5. Ribonuclease digestion of AU polymer (L. A. Heppel). Ultraviolet print of paper chromatogram (solvent system: 1.0 M ammonium acetate, 300 ml; 95% ethanol, 750 ml (15)). Bands at increasing R_F values correspond to penta-, tetra-, tri-, di- and mononucleotides, respectively. Spot at bottom center is a marker of uridylic acid (uridine-3′-phosphate).

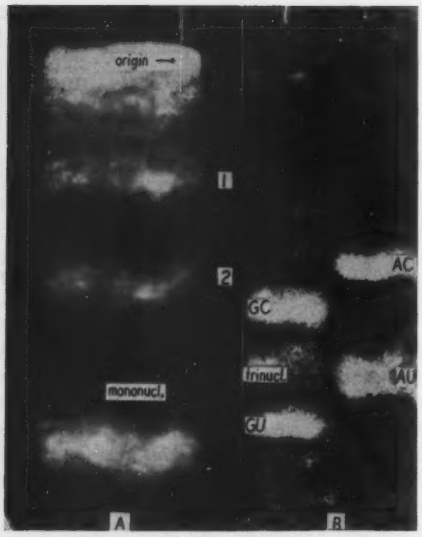

FIG. 6. Ribonuclease digestion of AGUC polymer (L. A. Heppel and J. D. Smith). Identification of products after Markham and Smith (16). Ultraviolet prints. A—Isopropanol-ammonia chromatogram; B—paper electrophoresis (20 v/cm, 2 hr., pH 3.5) of eluates of bands 1 (*left*) and 2 (*right*) of paper chromatogram. The bands marked GC, GU, AC and AU are in the positions corresponding to the dinucleotides guanylic-cytidylic, guanylic-uridylic, adenylic-cytidylic and adenylic-uridylic acid, respectively.

TABLE 1. NUCLEOTIDE COMPOSITION

(R. M. S. SMELLIE)

Polynucleotide	Adenylic Acid	Guanylic Acid	Uridylic Acid	Cytidylic Acid
Azotobacter RNA	10.0	13.0	7.3	9.0
AGUC polymer	10.0	5.7	7.3	10.0

isopropanol-hydrochloric acid solvent system and determined from the ultraviolet absorption of the eluates of the corresponding spots. The results are the averages of a number of closely-agreeing experiments. It is apparent that the proportions of adenylic, uridylic, and cytidylic acids were essentially the same in the two polynucleotides, whereas the proportion of quanylic acid in the biosynthetic polymer was about half that in *Azotobacter* RNA. It should be remembered that the AGUC polymer was prepared from a mixture of nucleoside diphosphates containing approximately equimolar amounts of adenosine, uridine and cytidine diphosphate but only about half the amount of guanosine diphosphate (3).

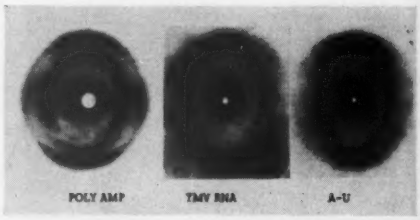

FIG. 7. X-ray diffraction patterns of fibers of tobacco mosaic virus RNA, and biosynthetic ribo-polynucleotides (A. Rich).

It appears from this that an AGUC polymer prepared from equimolar mixtures of the four nucleoside diphosphates might have a nucleotide composition very close to that of the RNA obtained from *Azotobacter* cells.

X-ray Diffraction Patterns. Some of the biosynthetic polymers can be stretched into fibers (19) from which Dr. Alexander Rich has obtained X-ray diffraction patterns suggesting a high degree of orientation (20). As shown in figure 7, single polymers, such as the A polynucleotide, give X-ray diffraction patterns which are very similar although not identical to those given by natural RNA. On the other hand, biosynthetic polynucleotides containing both purine and pyrimidine bases, give patterns virtually identical to those of natural RNA. In figure 7 the patterns of the A and AU polymers are shown together with that of a sample of tobacco mosaic virus RNA. Patterns similar to that given by the AU polymer have been obtained from AGUC.

PHYSICAL PROPERTIES

Ultracentrifugal studies by Dr. R. C. Warner in our laboratory suggest average molecular weights ranging from 50,000–350,000 for the biosynthetic polynucleotides. These values are in the range of molecular weights determined for RNA from different sources by various investigators. Higher values, namely 570,000 and 800,000 for an A and I polymer, respectively, were obtained by the method of light scattering (21). However, during the preparation of the latter polymers the equilibrium was shifted at various time intervals by supplementary additions of nucleoside diphosphate to promote further synthesis. It is possible that polymers of large size are obtained by multiple additions of nucleoside diphosphates.

In table 2 the approximate average molecular weights (sedimentation) of some biosynthetic polymers are compared with the molecular

TABLE 2. APPROXIMATE MOLECULAR WEIGHT (MW) AND CHAIN LENGTH OF BIOSYNTHETIC POLYNUCLEOTIDES

Polymer	Sedimentation data*		End-group Assay†	
	Homogeneity	Approx. MW	Av. chain length	Calc. MW
A	Monodisperse	70,000	230	70,000
U	Monodisperse	70,000		
A and U mixed	Monodisperse	300,000		
AU	Polydisperse	300,000	120	40,000
AGUC	Polydisperse	70,000	30	9,000

* R. C. Warner. † Data of J. D. Smith.

weights calculated from the average chain-length as determined by end-group assays. It will be seen that while there is no discrepancy between the two values in the case of the A polymer, there are wide discrepancies in the case of the AU and AGUC polymers. Here the molecular weights calculated from chain length are much smaller than those calculated from sedimentation.

The above discrepancies might be ascribed to branching of the polynucleotide chains but might also be due to aggregation of polynucleotide molecules. The latter is strongly suggested by the fact that when solutions of A and U polymer are mixed (table 2) a compound of much higher molecular weight is formed which sediments with a single boundary (22). The product of interaction appeared to be monodisperse as were the separate A and U polymers. On the other hand, both the AU and the AGUC polymers were polydisperse. Interaction between the A and U polymers was also disclosed in electrophoresis experiments (22) in which the product migrated as a single component with a mobility intermediate between that of the A and U polymers. Dr. Warner has also established the occurrence of interaction by the marked decrease in optical density occuring at the peak of ultraviolet absorption when the A and U polymers are mixed (22). Aggregation may occur through hydrogen bonding between appropriate pairs of bases as suggested for the double-stranded helical structure proposed for DNA by Watson and Crick (23) on the basis of x-ray diffraction measurements. The average chain lengths of the A, AU, and AGUC polymers were widely different. The value of 30 residues obtained for AGUC falls within the limits of 12 and 53 residues found by Markham and Smith (24) for the chain-length of samples of yeast and turnip yellow mosaic virus RNA, respectively.

DISTRIBUTION OF POLYNUCLEOTIDE PHOSPHORYLASE

Recent work indicates that polynucleotide phosphorylase is widely distributed in microorganisms. The enzyme has been found in extracts of a number of bacterial cells, whether aerobic or anaerobic, gram positive or gram negative (4). Data on the bacterial distribution of the enzyme are shown in table 3. It may be seen that *Azotobacter vinelandii*, the organism in which the enzyme was first found, is the richest source. High activity is also exhibited by extracts of *Staphylococcus aureus*, *Mycobacterium phlei* and *Micrococcus lysodeikticus*, as well as *Escherichia coli*, *Bacillus cereus* and *Clostridium kluyveri*. Not included in the table is the organism *Alcaligenes faecalis*, extracts of which are also very rich in polynucleotide phosphorylase. Dr. Manago has recently found the enzyme in yeast extracts (25). Among higher plants weak polynucleotide phosphorylase activity has so far been found in ammonium sulphate fractions of spinach leaves (4).

No unequivocal evidence has yet been obtained for the presence of the enzyme in extracts of animal tissues. Indirect evidence for its presence in liver may be provided by Goldwasser's finding (26) that AMP (5′-adenosine monophosphate) is incorporated as such into the RNA of pigeon liver homogenates. Similar results have been obtained by Heidelberger, Potter and collaborators with rat liver homogenates (27). Although these experiments do not show that adenylic acid is incorporated following its conversion to adenosine diphosphate, this would not appear unlikely as the experimental conditions favored the formation of adenosine polyphosphates by oxidative phosphorylation. In animal

TABLE 3. POLYNUCLEOTIDE PHOSPHORYLASE IN BACTERIAL EXTRACTS

(D. O. BRUMMOND)

	(Relative activity per mg. protein)
A. vinelandii	100
Mycobact. phlei	55
M. lysodeikticus	42
E. coli (Crooks)	25
E. coli (4157)	35
Staph. aureus (Duncan)	75
Strep. haemolyticus	15
Strep. faecalis	2
Strep. lactis R.	10
Pneumococcus (type III)	7
B. cereus	25
Cl. kluyveri	25
Corynebact. diphtheriae	5
Cl. acetobutylicum	not detected
L. arabinosus	not detected

tissues the enzyme might be present in very small amounts. It would not be surprising that rapidly proliferating cells would contain larger amounts of polynucleotide phosphorylase than cells that do not multiply rapidly.

CONCLUDING COMMENTS

Present information on structure, size, x-ray diffraction pattern, and behavior toward different enzymes indicates that the polynucleotides synthesized by polynucleotide phosphorylase from 5′-nucleoside diphosphates are closely related to RNA. Indeed the AGUC polymer, i.e. the one containing adenylic, guanylic, uridylic, and cytidylic acid residues, appears to be indistinguishable from native RNA. The available evidence indicates that these polynucleotides consist of 5′-nucleoside monophosphate units linked to one another through 5′-, 3′-phosphoribose diester bonds and that the chains are terminated by a phosphate group esterified at carbon 5′ of the nucleoside moiety. This is what would be expected from the fact that the biosynthetic polynucleotides are polymers of 5′-nucleoside monophosphate units resulting from the splitting of orthophosphate from 5′-nucleoside diphosphates. As already mentioned the polynucleotide chains of certain plant viruses have the same structure but in other cases (yeast RNA, turnip yellow mosiaic virus RNA) the chains appear to be terminated by a phosphate group esterified at carbon 3′ of the nucleoside moiety (14, 24). However, as pointed out by Brown and Todd (12), it is difficult to decide whether this was the structure of the intact polynucleotide chain in the cell since nucleic acids are subject to degradation by enzymatic or chemical action during extraction. It may be seen on inspection of the diagrams of figures 2 and 4 that such degradation, if due to ribonuclease, to phosphodiesterases of the spleen type, or to alkali or acid (28) would lead to chains terminated by a phosphate group esterified at carbon 3′ of the ribose moiety.

Smellie's data on the nucleotide composition of the AGUC polynucleotide suggest that if the polymer were made from equimolar concentrations of the four nucleoside diphosphates, its nucleotide composition would be identical to that of *Azotobacter* RNA. It may be added that the analogy between *Azotobacter* RNA and the AGUC polymer synthesized by the *Azotobacter* enzyme is also found with regard to molecular weight. Measurements by Dr. Warner gave a sedimentation constant for *Azotobacter* RNA only slightly lower than that of the AGUC polynucleotide.

In view of the fact that ribonucleic acids appear to be involved in protein synthesis and of the widely held idea that they may provide templates for the synthesis of specific proteins, the problem as to the specificity or otherwise of RNA and the manner in which specific nucleic acids could be synthesized is of considerable importance. The only case where specificity of RNA is well established is in plant viruses. Magasanik (29) has emphasized the fact that different viral species contain RNA of characteristically different composition while different strains of the same species possess RNA of identical nucleotide composition. Furthermore, even ribonucleic acids of the same nucleotide composition may exhibit specificity of function. This has been elegantly illustrated by recent work of Fraenkel-Conrat (30) showing that hybrid tobacco mosaic virus, reconstituted from protein and RNA components of different strains, produced lesions characteristic of the strain possessing the RNA present in the reconstituted virus. Moreover the hybrid viruses yielded a progeny containing both the protein and the RNA of the strain from which the RNA in the mixed virus was derived.

The question whether enzymes similar to polynucleotide phosphorylase are concerned with the synthesis of deoxyribonucleic acids is of great interest in view of the specific genetic functions of DNA (31). No answer can as yet be given to this question although the problem is under investigation in several laboratories. It has been shown (32, 33) that the 5′-di- and triphosphates of deoxyadenosine, deoxycytidine, and thymidine are formed in liver homogenates by phosphorylation of the corresponding 5′-nucleoside monophosphates. Drs. Brummond and Smellie (unpublished) have recently found that ammonium sulfate and ethanol fractions prepared from *Azotobacter* extracts contain enzymes catalyzing the transfer of phosphate from ATP or ITP to 5′-deoxyadenosine and 5′-deoxycytidine monophosphate; both deoxyribonucleoside di- and triphosphates are formed. These reactions appear to involve phosphate transfer from ATP. The occurrence of the mono-, di-, and triphosphates of thymidine and deoxycytidine in thymus extracts has also been reported (34). These observations would lend support to the view that the deoxyribosnucleoside diphosphates may be precursors of DNA. Recent observations or

Kanazir (35, 36) may also be of significance in this connection. Ultraviolet irradiation of *E. coli*, a treatment which results in an immediate and complete block of DNA synthesis, led to accummulation of ATP and other acid-soluble products in the cell including thymidylic, and possibly deoxyguanylic and deoxycytidylic acids. These results would implicate the deoxyribonucleotides as precursors of DNA if it be assumed (37) that blockage of DNA synthesis leads to accumulation of its more immediate precursors.

More direct support for the involvement of 5′-deoxyribonucleoside polyphosphates in DNA biosynthesis is provided by recent studies of Kronberg *et al.* (38). These workers have reported the formation of a thymidylic acid polynucleotide on incubation of *E. coli* enzyme fractions with C^{14}-thymidine and ATP; the reaction appears to involve 5′-thymidine polyphosphates. The operation of a similar mechanism *in vivo* is suggested by the finding of Price *et al.* (39) that when *E. coli* is grown in the presence of 5-bromouracil (a thymine analog) and P^{32}-orthophosphate, the specific P^{32}-activity of the 5′-bromouridylic acid isolated from the DNA is as high as that of the intracellular orthophosphate itself. This result is interpreted to mean that 5-bromouracil is incorporated into DNA as deoxybromouridine-5′-phosphate.

Little is as yet known of the mechanism of action of polynucleotide phosphorylase. The question of whether the enzyme, in analogy with muscle phosphorylase, can only add nucleotide units to a pre-existing polynucleotide chain, i.e. whether the enzyme requires a primer, or whether it can build a polynucleotide chain starting from a mixture of nucleoside diphosphates is undoubtedly of interest. No definite answer can yet be given to this question and studies aimed at elucidating this point may have to await further purification of the enzyme.

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STUDIES ON STRUCTURE OF RIBONUCLEASE

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The present report will be limited to a review of the main steps in the study of the structure of ribonuclease and the results that have been obtained thus far. In a brief talk we cannot acknowledge in detail the stimulus which this research has received from the several structural studies on peptides and proteins which have been completed within the past few years. These investigations include, of course, the classic work of Sanger and his associates on insulin, and the researches of du Vigneaud and his colleagues on oxytocin and vasopressin, Craig and associates on antibiotic peptides, and Bell *et al.*, White and Landmann, and Li *et al.* on corticotropins.

Preparation of Ribonuclease. A relatively heat-stable enzyme capable of digesting yeast nucleic acid was shown to be present in pancreatic extracts by Jones in 1920 (1). Dubos and Thompson (2) partially purified the enzyme by the use of heat-denaturation and acetone precipitation to remove less stable proteins. Kunitz (3) isolated the protein in crystalline form from beef pancreas in 1940, primarily by means of fractional precipitation by $(NH_4)_2SO_4$. The enzyme proved to be a small protein with a molecular weight of about 15,000 and an isoelectric point near pH 8.

With the advent of improved chromatographic techniques, ribonuclease was one of the first proteins to be examined chromatographically. Martin and Porter (4) in England employed liquid-liquid chromatography, and Hirs, Moore, and Stein (5, 6) examined preparations on columns of a polycarboxylic acid resin, IRC-50. Both techniques were capable of removing detectable amounts of impurities from crystalline ribonuclease prepared by the method of Kunitz (3) and McDonald (7). Evidence was also obtained for the occurrence of a small amount of another ribonuclease in pancreas (4, 6). The predominant enzyme, ribonuclease A, was prepared in chromatographically homogeneous form (fig. 1), and the amino acid composition of the protein was determined using this sample (8). Subsequent structural work was done on commercially pre-

pared material which assayed at least 93% ribonuclease A by chromatographic analysis.

Amino Acid Composition. The analyses (8) were performed upon 2 mg samples hydrolyzed for 20 hours and 70 hours in order to obtain, by extrapolation to zero time, corrected values for those amino acids that decomposed measurably under the hydrolytic conditions. The hydrolysates were analyzed by chromatography on columns of Dowex 50-X4 (9), as illustrated in figure 2. Cystine was determined independently as cysteic acid by the procedure of Schram, Moore and Bigwood (11). The protein does not contain detectable quantities of tryptophan. In general, the amino acid analyses by the chromatographic methods were in good agreement with the earlier findings of Brand (12), except for leucine and alanine. Assuming a molecular weight of about 14,000, integral molar ratios were obtained for most of the amino acids.

The analytical results (8) have had to be revised slightly in the light of subsequent structualr studies (10), which have indicated that the corrections that were applied to compensate for the decomposition of proline and aspartic acid led to values which were high by one residue each. It is now quite certain that the empirical formula for ribonuclease (table 1) in terms of 124 amino acid residues is: Asp_{15} Glu_{12} Gly_3 Ala_{12} Val_9 Leu_2 $Ileu_3$ Ser_{15} Thr_{10} $(Cys\text{-})_8$ Met_4 Pro_4 Phe_3 Tyr_6 His_4 Lys_{10} Arg_4 $(—CONH_2)_{17}$. This formula leads to a calculated minimum molecular weight of 13,683.

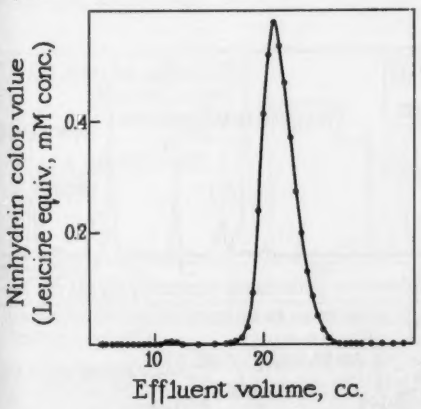

FIG. 1. Chromatographic homogeneity of ribonuclease A prepared by passage over IRC-50 (6). Sample, ca. 8 mg. Column, 0.9 x 30 cm. IRC-50 buffered at pH 6.47 (0.2 M sodium phosphate).

Several attempts to confirm the reported (13) presence of sulfhydryl groups in ribonuclease have not been successful (14–18). It is assumed, therefore, that four disulfide bridges are present, as postulated in 1954 by Anfinsen, Redfield, Choate, Page and Carroll (14), who also demonstrated, by the dinitrophenyl technique of Sanger (19), that ribonuclease consists of a single peptide chain with the sequence Lys.Glu.Thr.Ala- at the amino-terminal end. You will note several references in this discussion to the work of Dr. C. B. Anfinsen and his associates in Bethesda. Our respective laboratories have been aiming at some of the same answers using somewhat different approaches, and it has been both profitable and pleasurable to share our findings as we go along.

Cleavage of —S—S— Bridges by Oxidation. Following the general procedure utilized by

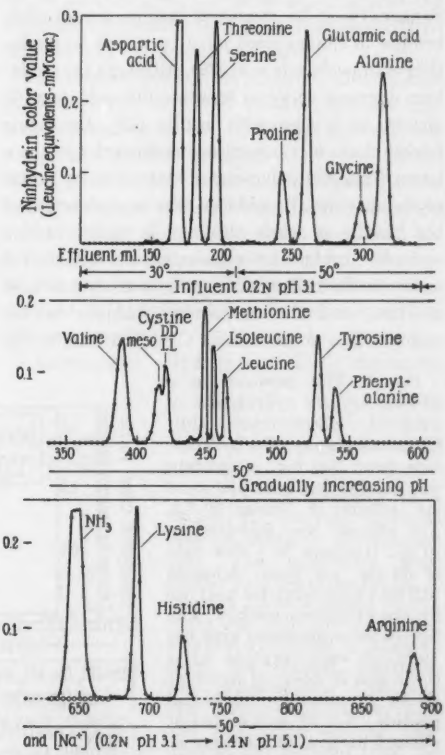

FIG. 2. Amino acid composition of an acid hydrolysate of ribonuclease A chromatographed on a 0.9 x 150 cm. column of Dowex 50-X4 (8, 9). The sample was 2 mg. of protein, hydrolyzed for 70 hours with 0.5 ml. of 6 N HCl.

TABLE 1. AMINO ACID COMPOSITION OF RIBONUCLEASE*

Amino Acid	No. Residues per molecule (m.w. 13,683)	Amino Acid	No. Residues per molecule (m.w. 13,683)
Aspartic acid	15	Methionine	4
Glutamic acid	12	Proline	4
Glycine	3	Phenylalanine	3
Alanine	12	Tyrosine	6
Valine	9	Histidine	4
Leucine	2	Lysine	10
Isoleucine	3	Arginine	4
Serine	15	Amide NH_3	(17)
Threonine	10		
Half-cystine	8	Total number of residues	124

* Ribonuclease from beef pancreas; from (9), as revised in (10).

Sanger (19) in his studies of insulin, the disulfide bridges in ribonuclease have been split by oxidation with performic acid (20). Although the oxidation does not serve to separate different peptide chains, as it does with insulin (19), the single folded chain of ribonuclease is opened up into a linear form which facilitates hydrolysis by proteolytic enzymes. In addition, the interpretation of the results of such cleavage is easier, if the —S—S— bridges are eliminated. Performic acid converts the half-cystine residues to cysteic acid residues, and the methionine residues to the sulfone. The abbreviations Cys- and Met in the following discussion refer to these oxidized forms. No other amino acids are affected by the oxidation, although precautions are required to prevent alteration of tyrosine (20, 21). Ribonuclease, fortunately, does not contain tryptophan, which would be oxidized by performic acid, and further study is required before it will be possible to rupture specifically the disulfide bridges in proteins that contain tryptophan.

Tryptic Hydrolysis. As the first step in the determination of the sequence of amino acids in the A and B chains of insulin, partial hydrolysis by HCl was utilized by Sanger and Tuppy and Thompson (22, 23). As Sanger has pointed out (19), however, it is doubtful whether random partial hydrolysis by concentrated HCl could be used effectively with much larger molecules. The complex mixture of peptides which would result from the partial acid hydrolysis of a protein of the size of ribonuclease would be very difficult to fractionate, and deduction of a unique sequence for the parent molecule becomes increasingly difficult as the number of small peptides in the partial hydrolysate increases. Proteolytic enzymes, on the other hand, because of their specificity, are capable of cleaving a protein into a limited number of fragments. Enzymes were used by Sanger and his associates (22, 23) for the elucidation of parts of the structure of insulin, and have subsequently been employed by Bell (24), White and Landmann (25) and Li *et al.* (26) in their investigations of the structure of ACTH.

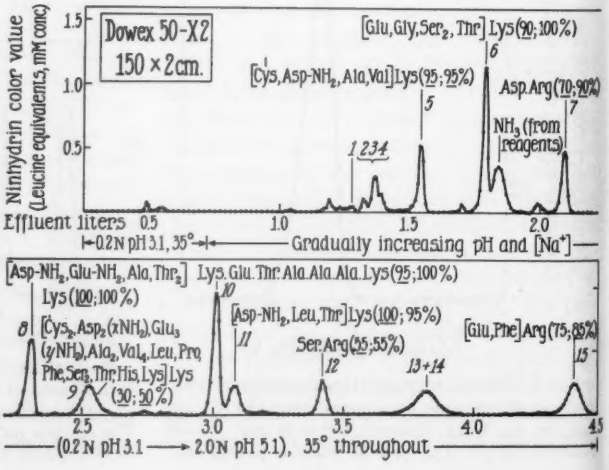

FIG. 3. The peptides in a 20-hour tryptic hydrolysate of oxidized ribonuclease (10). Chromatography of a hydrolysate from 200 mg. of protein was carried out on a 150 x 1.8 cm. column of Dowex 50-X2. The effluent was collected in 10 ml. fractions at a flow rate of 30 ml. per hour. Aliquots (0.5 ml.) were taken for analysis by the ninhydrin method. The figures in parentheses give the yield of each peptide after three and 20 hours of hydrolysis by trypsin. An additional peptide (No. 16) was eluted at about 5 liters after a change at 4.5 effluent liters to 2 N sodium acetate buffer at pH 6.6, and a change in temperature to 50°. The abbreviation Cys- represents a cysteic acid residue in the peptides from the oxidized protein.

Of the readily available proteolytic enzymes, trypsin possesses the sharpest specificity, as Bergmann, Fruton and Hofmann (cf. 27) demonstrated by the use of synthetic substrates. Trypsin might be expected to hydrolyze oxidized ribonuclease at the peptide bonds involving the carboxyl groups of the 10 lysine and 4 arginine residues present in the molecule (table 1) to yield 14 or 15 fragments. Whether or not free lysine would be cleaved from the amino-terminal position could not be foretold with assurance.

Accordingly, oxidized ribonuclease was hydrolyzed by trypsin at pH 7.0 (10), and the products formed were separated by ion exchange chromatography on columns of Dowex 50-X2 (fig. 3). These columns permit the isolation of 10–50 mg quantities of peptides, which can be rendered salt-free, if necessary. As a first step, quantitative amino acid analyses were performed on an aliquot (about 1/10th) of the material in each peak, and the results were particularly informative in deciding whether or not a single peptide was present. A reasonably well purified peptide should contain only a limited number of amino acids in quantities that give integral molar ratios. When more complex mixtures are chromatographed, it may become necessary to employ paper chromatography or zone electrophoresis as additional tests of homogeneity. The amino acid analyses were originally performed by the method previously described (9, 20). At present, automatic recording equipment described at this meeting by Spackman, Stein and Moore (28) is being used.

The amino acid analyses showed that mixtures of peptides were present in the zones marked 2, 3, 4, and 13 + 14 (fig. 3), and upon rechromatography, four additional pure peptides were obtained. A fifth, peptide 16, referred to in the legend to figure 3, contained 20 residues but was devoid of lysine or arginine, and therefore represented the segment liberated from the carboxyl-terminal portion of the chain. In all, 13 principal peptides were isolated which accounted exactly for a molecule of 124 amino acid residues. Two lysyl bonds were not split by trypsin, one at the amino terminal position (peptide 10), and one in peptide 9, which may be a trypsin-resistant —lysylprolyl— bond (cf. 24). Peptide 10 serves to extend the known sequence at the amino-terminal end to seven residues (10). The percentage yields of each of the peptides per molecule of ribonuclease (cf. fig. 3) were calculated from the amino acid analyses on the assumption that the sequence represented by a given peptide occurred only once in the protein molecule.

The compositions of the peptides, and the high yields in which they were isolated, indicate that transpeptidation is not occurring and will not complicate the interpretation of the results obtained when trypsin is used as a reagent for the specific cleavage of the protein. To determine the order in which these 13 peptides are linked together, it is clearly necessary to learn the nature of the products formed when oxidized ribonuclease is hydrolyzed by an enzyme with a different specificity.

Chymotryptic Hydrolysis. The chromato-

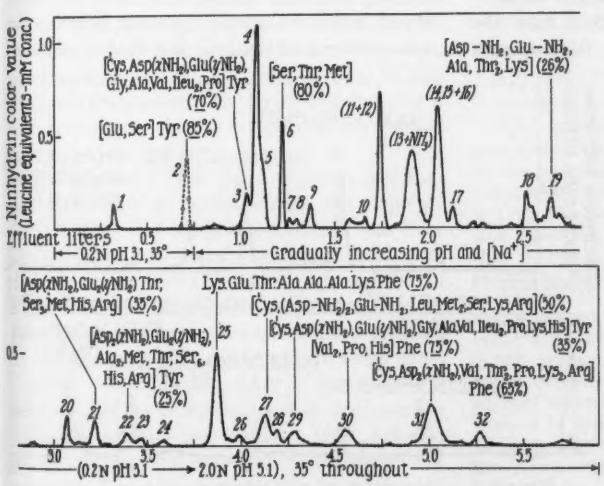

FIG. 4. The peptides in a 24-hour chymotryptic hydrolysate of oxidized ribonuclease (29). The conditions were similar to those given for fig. 3. Peptide 2 was detected by the ninhydrin reaction only after alkaline hydrolysis of aliquots from the effluent fractions. The abbreviations Cys- and Met represent cysteic acid and methionine sulfone residues in the peptides from the oxidized protein.

graphic separation of the peptides liberated by the action of chymotrypsin is illustrated in figure 4 (29). The enzyme is less specific than trypsin, and the resulting mixture is more complex. Bergmann and Fruton (cf. 30) showed that chymotrypsin hydrolyzes preferentially peptide bonds involving the carboxyl groups of phenylalanine and tyrosine, and this fact is borne out by the data in figure 4. Amino acid analyses indicated the presence of mixtures of peptides in the regions of the curve marked 4 + 5, 11 + 12, and 14 + 15 + 16. These were separated by rechromatography under different conditions of pH and temperature (29).

In all, 18 purified peptides, containing from 3 to 27 amino acid residues each, were isolated from the chymotryptic hydrolysate in yields of from 6 to 80%. The 6 tyrosine residues and the 3 phenylalanine residues shown by analysis to be present in the protein were obtained in eight peptides isolated in good yield. One of the peptides contained two tyrosine residues and also a proline, indicating that the chymotrypsin resistant tyrosyl bond might be —Tyr.Pro—. Because the 18 peptides obtained add up to considerably more than 124 residues, some of the fragments isolated in low yields must represent overlapping peptides from the same section of the ribonuclease chain. For reasons such as these, the data obtained after chymotryptic hydrolysis are more difficult to interpret than were those secured after the action of trypsin. The results provide, however, the principal information which makes it possible to determine the order in which the 13 segments obtained by tryptic hydrolysis are linked together.

Peptic Hydrolysis. Additional data have also been secured through the use of a third enzyme From the mixture obtained by the hydrolysis of oxidized ribonuclease by pepsin, eight peptides have been separated in yields of 10–100% by Bailey *et al.* (31; fig. 5). However, less than 60% of the 124 amino acid residues in the protein have been accounted for in terms of two tetra-, two penta-, one hexa-, and one octapeptide, and two larger fragments of 12 and 43 amino acid residues. The largest peptide was isolated by chromatography on IRC-50 (31) rather than on Dowex 50-X2. Pepsin appears to cleave a considerable number of peptide bonds in ribonuclease, and is less specific than is either trypsin or chymotrypsin. Taken in conjunction with the other data, however, the results obtained with pepsin have been helpful in the derivation of the structure of several sections of the ribonuclease molecule.

A Partial Structural Formula. A consideration of the findings discussed above has made it possible to propose as a working hypothesis a formulation that shows the manner in which the peptides produced by the action of trypsin, chymotrypsin and pepsin are joined to one another in the original molecule of oxidized ribonuclease (29). In the derivation of this scheme (fig. 6), certain assumptions have been made. The amino acid residues in oxidized ribonuclease are presumed to be linked in a single unbranched chain containing peptide bonds between only α-amino and α-carboxyl groups. The evidence for a single chain has been presented by Anfinsen *et al.* (14), and there is thus far no evidence that branching occurs. It has also been assumed that in peptides resulting from the specific enzymatic action of trypsin, lysine or arginine, if present, occupies the .carboxyl-terminal position, and that tyrosine or

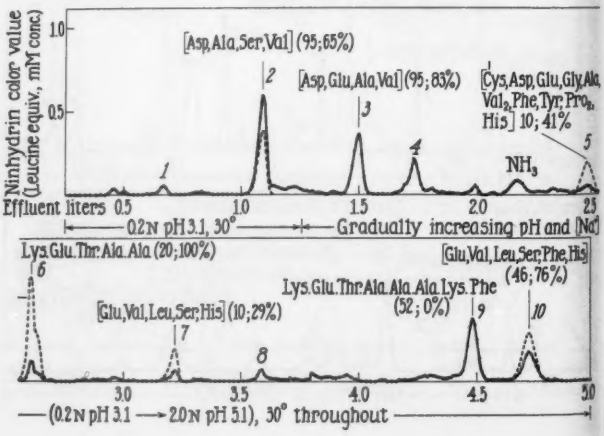

FIG. 5. The peptides in a 4-hour peptic hydrolysate of oxidized ribonuclease (31). The peaks shown by the dashed line were obtained from a 24-hour hydrolysate. The conditions were similar to those given for fig. 3. A peptide containing 43 residues (No. 11) was isolated by chromatographing the hydrolysate on IRC-50 (31).

phenylalanine, if present in peptides formed by the action of chymotrypsin, is carboxyl-terminal. If these assumptions are incorrect, this should become apparent during the derivation, for it should not then be possible to derive a unique formula consistent with all of the experimental data. For convenience, the numbered peptides from the three series of experiments on the oxidized protein are preceded by the abbreviations O-Tryp, O-Chy, and O-Pep. Residues in parentheses are of undetermined order.

In deriving the arrangement shown in figure 6, it is simplest from the point of view of exposition to start at the amino-terminal end of the chain and to proceed stepwise toward the carboxyl-terminal end, linking, in appropriate order, the thirteen peptides obtained by tryptic hydrolysis.

First of all, it is known that the heptapeptide sequence Lys.Glu.Thr.Ala.Ala.Ala.Lys.—(O-Tryp 10) occurs at the amino-terminal end of the molecule (10, 14). An octapeptide corresponding in amino acid composition to O-Tryp 10 plus one residue of phenylalanine has been isolated from both the chymotryptic (O-Chy 25) and peptic (O-Pep 9) hydrolysates. From the composition of O-Chy 25, and O-Pep 9, phenylalanine must occupy the eighth position in the chain. The second O-Tryp segment in the sequence must, therefore, contain a phenylalanine residue in the amino-terminal position. There are only three phenylalanine residues in ribonuclease, which are present in O-Tryp 9, 15, and 16. Because it contains no lysine or arginine, O-Tryp 16 must be the carboxyl-terminal section of the molecule and cannot follow O-Tryp 10. In O-Tryp 9, phenylalanine has been found not to be amino-terminal by the DNP-technique of Sanger, so that by elimination, the tripeptide O-Tryp 15 follows O-Tryp 10, and the known sequence at the amino-terminal end of the ribonuclease chain is extended to ten residues by —Phe.Glu.Arg—.

The peptide of the chymotrypsin series that follows O-Chy 25 must contain the sequence —Glu—Arg— at the amino-terminal end. Four O-Chy peptides contain glutamic acid and arginine residues, O-Chy 14, 21, 22, and 27. Of these four possibilities, two can be eliminated by showing that, if they were placed in this position, no remaining peptide of the trypsin series could possibly follow directly after O-Tryp 15. For example, O-Chy 27 is a decapeptide that contains lysine in addition to arginine. If it followed O-Chy 25, it would have to extend through one complete lysine-containing O-Tryp peptide of eight or less residues (O-Tryp 5, 6, 8, 11, or 14), or one of the two lysine residues of the longer peptide O-Tryp 9. Simple consideration of the quantitative amino acid composition of each of the peptides concerned shows that none of these combinations is permissible. O-Chy 14 can be excluded on similar grounds, leaving only O-Chy 21 and 22 as possibilities. These two peptides, obtained in yields of 35 and 25% respectively, contain 10 residues in common, and it has been concluded that they come from the same portion of the chain (29). Accordingly, they are placed in the manner indicated in figure 6.

The presence of six serine residues in O-Chy 22 requires the same number in the peptide of the O-Tryp series that follows O-Tryp 15. O-Tryp 4 is the only peptide fulfilling this requirement and must be the next segment in the chain.

In a similar manner, which time does not permit us to set forth here, by alternating between the peptides of the trypsin and the chymotrypsin series, it has been possible to extend the analyses systematically to the end of the chain (26). The independent data of Anfinsen and Redfield (32) on the occurrence of the sequence —Leu.Thr.Lys.Asp.Arg— have helped to establish the sequence of nine residues embracing O-Tryp 12, 11, and 7, which otherwise would have required additional amino-end group analyses. When we reach the carboxyl-terminal segment of the molecule (O-Tryp 16), the data of Anfinsen and his associates have again been used to assign O-Chy 4 to the end position. They found, by the use of carboxypeptidase, that valine is the carboxyl-terminal residue (14), and also showed that the terminal tetrapeptide had the structure —Asp.Ala.Ser.Val (32).

A tentative placement[1] of amide groups has

[1] The tentative nature of the allocation of glutamine and asparagine residues is emphasized by the fact that on the basis of further considerations, the amide group initially attributed to O-Tryp 15 (10, 29) has been deleted in figure 6. The stabilities of O-Chy 21 and 22 indicate that they probably do not contain an amino-terminal glutamine residue, which would tend to undergo cyclization to a ninhydrin-negative pyrrolidonecarboxylic acid derivative, as has been found to occur with O-Chy 2 (fig. 4). In addition, subsequent preparations of O-Tryp 15 have given nearly NH_3-free acid hydrolysates. The probable number of amide groups per molecule has been kept at 17 (table 1) by increasing the number of amide groups in O-Tryp 9 to 4, in line with the analytical results obtained with that peptide (10).

Lys.Glu.Thr.Ala.Ala.Lys.Phe.Glu.Arg.(Asp,Glu,Thr,Ser3,Met,His).(Asp,Ala2,Ser3).Tyr.(Cys,Asp,Glu,Met2).Lys.Ser.Arg.Asp.Leu.Thr.Lys.
O-Chy 25
O-Chy 21
O-Chy 11
O-Chy 27
O-Pep 6
O-Chy 22
O-Pep 9
O-Pep 11
O-Tryp 15
O-Tryp 10
O-Tryp 4
O-Tryp 12
O-Tryp 11
—Asp.Arg.(Cys,Asp,Val,Pro,Thr,Lys).Phe.(Glu,Val,Leu,Ser,His).(Asp,Glu,Ala,Val).(Ser,Cys,Glu,Ala,Val).Lys.Asp.(Cys,Ala,Val).Lys.—
O-Chy 31
O-Chy 16
O-Chy 12
O-Pep 3
O-Pep 10
O-Pep 7
O-Tryp 9
O-Tryp 5
O-Tryp7
—(Cys,Asp,Asp,Glu,Gly,Thr).Tyr.Glu.Ser.Tyr.(Thr,Ser,Met).(Cys,Asp,Ileu,Thr,Ser).Arg.(Glu,Gly,Thr,Ser2).Lys.(Cys,Asp,Ala,Tyr,Pro).Tyr.Lys.—
O-Chy 5
O-Chy 2
O-Chy 6
O-Chy 14
O-Tryp 2
O-Tryp 6
O-Tryp 14
—(Asp,Glu,Ala,Thr2).Lys.His.(Val,Ileu2).(Cys,Asp,Glu,Gly,Ala,Pro).Tyr.(Val2,Pro,His).Phe.(Asp,Ala,Ser).Val
O-Chy 19
O-Chy 29
O-Chy 3
O-Chy 30
O-Chy 4
O-Pep 5
O-Pep 2
O-Tryp 8
O-Tryp 16

been undertaken by attributing to each peptide in figure 6 the number of amide groups corresponding approximately to the quantity of NH_3 found in the hydrolysates of the individual peptides of the trypsin and chymotrypsin series (29).

It should be emphasized that the formula shown in figure 6 is a working hypothesis, although it accommodates satisfactorily all of the data that have been accumulated. None have been omitted. Strong support for the scheme is provided by the results of experiments in which the large peptides of the trypsin series, O-Tryp 4, 9, 2, and 16, have been hydrolyzed by chymotrypsin. The sub-fragments formed were separated chromatographically, and their amino acid composition determined as before. The results were in agreement with those to be expected on the basis of the scheme shown in figure 6. For example, three peptides were obtained by chymotryptic hydrolysis of O-Tryp 4. One was identical in chromatographic behavior and amino acid composition to O-Chy 11. The other two had the composition Asp, Glu, Ser_3, Met, His, and Cys, Asp, Glu, Met_2, Lys, respectively.

Completion of the formula requires working out the sequence of each set of residues in parentheses, none of which is larger than eight residues. This task is currently being undertaken by the use of chemical and enzymatic methods.

Before a complete structural formula for native ribonuclease can be written, it is also necessary to establish the position of the four disulfide bridges now thought to be present in the native protein. The problem is similar to that solved for insulin by Sanger and his associates (33). In order to permit the isolation of cystine peptides, the behavior of the unoxidized protein towards proteolytic enzymes is being studied. The products of the cleavage of the active enzyme are also being investigated in several laboratories, in order to determine whether any portion of the molecule can be shown to be directly concerned with its catalytic action on ribonucleic acid. The partial structural formula in its present form is helpful in such endeavors, and also in research on the physical structure of the protein.

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Fig. 6. A partial structural formula for oxidized ribonuclease (29). The amino acids are arranged in a single chain of 124 residues, the formula for which has been derived from the results of the studies of the peptides liberated by tryptic, chymotryptic and peptic hydrolysis. The sequences of the amino acid residues in parentheses are undetermined. In the oxidized protein each half-cystine residue in the original protein is present as a cysteic acid residue, and each residue of methionine as the sulfone. The $—NH_2$ symbol above an aspartic or glutamic acid residue indicates the tentative allocation of an asparagine or glutamine residue to this position. The points of cleavage by trypsin are indicated by the solid line, of chymotrypsin by the dashed line, and of pepsin by the dotted line.

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AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS

Symposium on Teaching Practices in Biochemistry[1]

Chairman: J. MURRAY LUCK

INTRODUCTORY REMARKS

IT IS IMPROBABLE that any symposium on the teaching of biochemistry would fail to consider the Report of the First Teaching Institute of the Association of American Medical Colleges. The Institute met here in Atlantic City in October 1953 and concerned itself with physiology, biochemistry and pharmacology. Some of us who are here today had the pleasure and stimulating experience of participating in the work of the Institute. Valuable as was the Institute, I am certain that none of us harbored the illusion that it answered all the questions which concern the teaching of biochemistry. It was oriented definitely toward medical education and studied essentially the place of biochemistry and of two of the closely related preclinical sciences in the medical school and their relationships toward each other.

But the place of biochemistry in the scheme of things reaches out beyond the medical school. A goodly number of students from the biological sciences and from chemistry come to us year after year as undergraduates or as candidates for advanced degrees. Others are interested vocationally in the curricula of schools of agriculture, of veterinary medicine, and of nutrition and public health. Does this mean that our academic institutions must be prepared to multiply their departments of biochemistry? Does it mean that more and more curricula must be prepared and that special kinds of biochemistry must be taught to meet the presumed needs of students with different backgrounds of training and with different vocational objectives? Does this mean that biochemistry must continue to be dominated both administratively and, perhaps, in its teaching philosophy by schools of medicine? Has the time come for a new orientation of biochemistry within our universities—an arrangement both administrative and instructional which permits and encourages the presentation of the subject matter as that of a pure science? May this be accomplished without failure to do justice to our responsibilities to the applied fields? It is worthy of note "that physiological chemistry as started at Yale was not primarily connected with a medical school, but was an integral part of a general biological course without limitation on the direction or character of its possible development.[2]

The crux of this perennial problem is always to so improve our teaching and our definition of the subject matter that the biochemists who emerge from our universities may have a top quality training for this ever-new world. If their ultimate interests direct them principally toward research, they must have developed a solid foundation in chemistry and in one or more of the cognate sciences. They must have had the thrill of discovery and the invaluable experience of experiment design. If they are to practice medicine, the students' training in the subject must be such that the place of chemistry and of biochemistry in diagnosis, in internal medicine, in surgery and in many of the medical specialties may never cease to be recognized and appreciated.

The important question of horizontal integration with the other pre-clinical sciences, and of vertical integration with the clinical sciences can hardly escape consideration. This is an aspect of

[1] Atlantic City, April 18, 1956.

[2] CHITTENDEN, R. H. *American Society of Biological Chemists, 1906–1931.* Baltimore: 1945.

medical education which is fresh and provocative. Perhaps it is allied to another problem equally fascinating in its philosophy and its implications—that of project teaching. The experimental sciences are learned through the eyes and the fingers, and the value of laboratory instruction may hardly be overestimated. Project teaching has become increasingly popular in pharmacology and I hope that some of our participants in today's symposium may have cause to report upon its possibilities in biochemistry.

Whatever may be the facets selected for consideration by our speakers, I know that we shall be alerted to these and related problems which concern the teaching of biochemistry.

PRELIMINARY SURVEY OF THE TEACHING OF BIOCHEMISTRY IN THE UNITED STATES

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IN THE LAST FIFTY YEARS the subject of biochemistry has undergone profound changes. What effect have such changes had on the teaching of the subject?

This report describes the results of a preliminary survey made possible by a grant from the National Science Foundation. A more detailed discussion is to be found in a report submitted to the Foundation.

Altogether, some 17 institutions have been visited in New York, New Jersey, Connecticut and in Massachusetts. In terms of teaching, these institutions include the following schools: undergraduate, 8; graduate, 13; medical, 10; dental, 5; agricultural, 1; home economics, 2.

Judging by publishers' lists of biochemical texts which are in current use, it would be difficult to name a reputable undergraduate college in which some course in biochemistry is not offered. There is no graduate school of any consequence in which this is not true. And, of course, there are biochemical courses offered in medical, dental and agricultural schools, and schools of home economics.

As might be anticipated, the variations in content are considerable: from the most elementary to the most rigorous. There are considerable variations as to the qualifications of the staff: some as scholars are superb; a few excel as teachers. The physical equipment varies, too: in quite a number of places the latest devices used in instrumental analysis are abundant and represent an outlay of many thousands of dollars.

The Course. The course is the introductory one to the subject, whether at the undergraduate, professional or graduate level. Since many of the graduate courses in biochemistry are attached to the biochemical departments of the medical schools, and, since, as a rule, medical and graduate students are required to take the same introductory course, the survey becomes more limited.

It can be stated at the outset that the lecture topics do not vary very much, though, of course, the details will, depending upon the instructor, the time available, etc. There is a tendency in some quarters to depart from the sequence—historically and somewhat accidentally adopted—carbohydrates, lipids and proteins, and start a discussion with a study of the properties of the proteins. The reason advanced is that the proteins offer an opportunity for a discussion of physico-chemical principles which, a number believe, cannot come too early in the course. Furthermore, the proteins offer a stepping stone for a discussion of enzymes which, again, cannot be presented too early, since a study of the enzymes and enzyme mechanisms is at the heart of the subject itself.

When it comes to the matter of the laboratory, the variations in different institutions are considerable. In the first place, while there are several laboratory manuals on the market, relatively few instructors use them, except as reference books; instead, they prepare their own mimeograph sheets for students' use. And while some attempt is made to correlate the lectures with the laboratory work, this is often so difficult that some have abandoned the attempt entirely. Here the content is regulated by the staff (as, indeed, is largely true with the lectures).

Graduate students, particularly those regis-

tered for the Ph.D. degree, are relatively few; the numbers run from 5–12 at any one institution. And it is here at this level that much encouragement and stimulation must be given if the future of biochemistry is to continue to expand at an accelerated rate.

The requirement for admission to the introductory course in biochemistry is, as a rule, a one-year course in organic chemistry. Of course, organic chemistry presupposes general chemistry, and almost always the student has had some elementary course in biology. But nevertheless, the basic requirement for entrance is organic chemistry. Many of the instructors would like to see physical chemistry added to this requirement.

The Lectures. At the undergraduate level, the lectures are almost invariably given by one man. At the professional (and also graduate) level, the lectures are almost invariably given by several men—from 5 to 8 to 10, and more—representing members of the staff. In extensive discussions with staff members, it became clear that there were advantages and disadvantages to both systems. With but one lecturer, if he be a poor one, the course can be easily ruined, and prospective biochemists can be lost. Of course, if he is an able man who knows his work and has those mystic qualities which go under the name of 'inspirational', then the reverse is true. Furthermore, a certain continuity is obtained when just one man is responsible.

The other side of the picture is presented in some such way as this: biochemistry has become vast, and no one man can explore its many ramifications. In the professional schools there are usually six or more men on the staff, each of whom is a specialist in some one sector; why not make use of their qualifications? And indeed, why not give them an opportunity? As for the continuity being threatened, and, above all, as for the desire of each specialist to cover all he knows within a limited time, this can be largely overcome by encouraging—and sometimes requiring—all members of the department to attend one another's lectures, and by frequent conferences among the staff for the purpose of constructive criticism.

While the problem has not been solved to the satisfaction of all concerned, there is no question anywhere that, insofar as the graduate student is concerned, once he has completed his introductory course, he should be subjected constantly to the combined talents of the entire staff.

There is another problem which has become acute enough to raise its head fairly often. Everyone the author has spoken to is by now agreed that biochemistry is an important enough discipline to be included as an optional course for undergraduates, and to be offered either in the chemistry or in the biology department. (For various reasons, not always clear, in some colleges the course is given in the chemistry department; in others, in the biology department.) No science major, so the argument runs, can be considered well equipped without an introductory course in biochemistry. But what if the graduating student decides to become an M.D. or a D.D.S., instead of continuing his studies in chemistry or biology? As a prospective medical or dental doctor, he is compelled to take a course in biochemistry in his first professional year. Should he be discouraged during his undergraduate days from taking this subject, and perhaps take in its stead some more mathematics, or physics, or physical chemistry, or organic chemistry? Since many instructors in the professional schools are loath to allow credit for work done at the undergraduate level, is there not the danger of needless repetition and consequent waste of time?

The reaction to these questions is a mixed one. A number of instructors in the professional schools dismiss the problem by asserting that biochemistry as taught by them is a totally different affair from any biochemistry given in undergraduate schools. Others are of the opinion that they would prefer students without a previous course in the subject. All are agreed that a good background in mathematics and physics is desirable.

The content of the lectures may include carbohydrates, lipids, proteins, enzymes, digestion, blood, respiration, urine, intermediate metabolism, energy metabolism, foods, vitamins, tissues, detoxication, immunochemistry and antibiotics. Often, these last two topics are lightly touched upon, since they are taken up in detail under microbiology. In the agricultural school, blood and urine is excluded, and the stress is upon polysaccharides and plant products. In dental schools much time, relatively speaking, is devoted to saliva and to the chemistry of bone and teeth. The sequence of the topics is by no means uniform.

In two dental schools, the same lectures are offered to dental and to medical students. The laboratory work may differ in some details. Judging by discussions with various representatives, the feeling is growing that the two types of

students should come under the same biochemical roof.

It should be emphasized that a graduate student wishing to specialize in biochemistry may choose a biochemical department attached to a medical school, or one independent of it. Some institutions have no medical schools; here the choice is simple: whatever graduate work in biochemistry exists is to be found in the graduate school of arts and sciences, and in the school of agriculture. In most universities there are medical schools; and for various reasons, largely historic, the biochemical center is often found in the medical school, though other parts of the university may also engage in biochemical teaching and research. Under such conditions, the graduate student has the privilege of specializing under the supervision of a professor in the medical school or one outside of it.

In connection with the lectures, all the places visited also include conference hours (or 'recitation' hours), at which time discussions dealing with lectures—and laboratory—topics are undertaken. These discussions are usually in the nature of explaining the difficulties encountered by the student, rather than any attempt to 'quiz' the student.

Whereas the entire class in every school attends the lectures, the conferences are limited to not more than 12–15 students. In the laboratory, the entire class—often amounting to 100 or more students—may be accommodated at one time, provided the laboratory is large enough. When this is not the case, the class is divided into two or more sections depending upon the size of the laboratory. In any event, just as the conferences are limited to about 15, so, in the laboratory, provision is usually made so that there is an instructor for every 12–15 students.

The examinations are given periodically, usually some two or three during the term, and one final examination at the end of the semester. Rarely do the tests include practical examinations in the laboratory. This, in the opinion of some, is a shortcoming.

Perhaps the most prevalent drawback in the lectures, with the younger men, particularly, is the attempt to cover too much in too short a time. Sometimes the students were much too busy writing at top speed to appreciate to the full what was being said. And is it really necessary to cover so many pages of the note book? Is there not much that can be referred to a textbook? Are not a few stimulating ideas, plus the explanation of some obvious difficulties, more than enough in one discourse?

The Laboratory. If the lectures at different institutions bear some resemblance to one another, this is not true of the laboratory work. To be sure, some experiments, such as those involving quantitative determinations of blood constituents, are common in a number of colleges and universities; however, the philosophy underlying the course differs widely in different institutions.

The practical difficulties, which to some extent limit the value of the undergraduate course, more particularly, arise from limited space and limited apparatus, so that students often work in pairs rather than singly, and they perform different experiments to avoid the necessity of duplicating costly apparatus. But this situation is not unknown even in some of the professional schools.

While on the subject of laboratory work, it may be pointed out that almost invariably the instructor emphasizes the principles involved in the experiments about to be carried out; and during the laboratory hours which follow, the alert instructor has an excellent opportunity, by questions and discussions, to get to 'know' the students. It is, indeed, in the laboratory where instructor-student relationship may become of paramount importance.

Where dental students do not take the same course as medical students, the lectures and the laboratory work for these men tend to be along more traditional lines: the approach is that of a decade ago. The lectures, to be sure, do include some of the latest advances, but the laboratory work seems to have changed little over a period of time. What distinguishes the course is the emphasis on saliva, and a study of teeth and bone.

There is more and more agitation for doing what some schools already do, and that is to have the first two years (preclinical) the same for dental and medical students. "Far from subordinating dentistry to medicine," reads one report, "this concept recognizes that patients come to a dentist as entire organisms rather than disembodied mouths. It repairs the unnatural cleavage from general medicine which has led dentistry to study its special field in isolation for a hundred years."

The amount of animal work (whole animals, in contrast to tissue slices) varies. Some institutions have little. Others hold a number of demonstrations illustrating food deficiencies of one kind or another and involving the use of rats, rabbits,

guinea pigs, etc. Such experiments, in cooperation with groups of students, sometimes run through the better part of the term, and are an adjunct, so to speak, of the main laboratory activity.

The difficulties of correlating laboratory work with the lectures have already been stressed. In one institution, only too fully aware of this situation, a few fundamental experiments—each experiment elaborately designed—are selected to illustrate important statements made in the lecture. For example, the statement is made in the lecture that the liver forms urea; in the laboratory an experiment is set up to prove the point. Or again, statements are made in the lectures emphasizing glycolysis in the brain; or the part played by mitochondria in cell respiration. Here again, significant experiments would emphasize these statements. All such experiments are carried out by groups of students, and they get together to compare results.

Advanced Courses, Graduate Work. Relatively few places offer many formal advanced courses in the department of biochemistry. Two common courses deal with special biochemical preparations, and with biochemical techniques. Depending upon the institution and its requirements, the student may be directed to take one or more courses in a number of subjects: biophysics, microbiology, nutrition, etc.

A fairly well-equipped graduate, with substantial courses to his credit in the field of mathematics (through calculus), physics (at least one to two years), chemistry (quantitative, organic and physical) and biology (at least one year), will be expected to devote some three years toward acquiring his doctor's degree. Depending upon the particular department, and the university, he will undoubtedly take more courses in chemistry, perhaps some more in biology, and may be expected to pass a 'qualifying' or 'comprehensive' examination before he starts his more serious work in the laboratory. His particular mentor, as well as others of the instructional staff, can form a pretty good idea of the student's capabilities after watching him at work in the laboratory and in the seminar for some two years or so. The successful defense of the thesis, and the passing of a foreign language examination (usually German and French), may complete the requirements for the Ph.D.

It is at this level—during the three years of graduate work—that the student needs the guidance of the top men in the field of research. Here the teaching art is of secondary importance; of first importance is the creative mind. The tendency in the Germany of the past for students to wander to several universities to sit at the feet of pre-eminent scholars is not precisely followed in this country; but more and more the graduate student, with the advice of his college instructor, now selects the professor rather than the university.

The Staff. At some undergraduate schools, where the teaching is heavy—maybe 15 hours a week—and the classes are few and small in number, the instructor in biochemistry is often asked to teach one or more subjects outside his field of specialization. But this is not true of graduate and professional schools; here whatever teaching is done is in the field of biochemistry proper. In such places, the teaching—or, at least, the bulk of it—is so arranged that it is concentrated in one term, thus giving freedom for research in the following term. For it must be emphasized that aside from one's own proclivities, stimulus for research comes from the authorities in charge, who make it clear that publications are of the utmost importance in questions involving promotion. This is, of course, truer of universities (including the professional schools) than of colleges; here, too, research counts; but good teaching is also weighed in the balance.

The fact that frequent publications are demanded sometimes presents an acute problem to the younger man. He wants to do a good job at teaching, but his value is weighed in terms of research, so that the tendency may be to neglect the former in favor of the latter. This is, of course, not always true.

One distinguished professor has presented the situation in this way: I demand that every member of my staff be an effective teacher, because we owe this to the students; and our first job is to the students. If he is not a good teacher, he does not stay as an instructor, though he may stay as a research associate without teaching duties. However, no effective teacher can regard his position as secure unless he also produces creditable work in the field of research.

And, in fact, everyone with whom the topic was discussed agrees that the combination of effective teaching and active researcher is essential for the equipment of the *superior* teacher, though neither one in itself is enough.

As to the nature of the research problem undertaken by the younger staff member, he is usually given every opportunity to select his own topic

and to develop it individually (or as the leader of a group). In some cases he joins forces with the head of the department, particularly when the problem is 'big' and the results may be such as to arrest general attention. Here the pattern has been set in the past by German university professors. And, of course, it must be admitted that —to pick a name at random—Emil Fischer could not have accomplished all he did except for an army of assistants and Ph.D. candidates.

Students. How do the students react toward the course in biochemistry? How close is the contact between the student and the instructor? Are students asked to comment on the course? Undergraduate and graduate students usually have a different approach to the subject than do professional students; the former may take the course if they wish to; the latter *must* take it. The tendency in the medical school is to make the subject more and more a basic course in science, rather than one full of clinical applications. The reason for this is two-fold: in his first year the student is not prepared to understand fully the clinical implications; and the science itself, resting largely upon the shoulders of chemistry, physics and biology, has so broadened that no time is left in an introductory course to stress applications; though, of course, some obvious allusions to pathology (carbohydrate metabolism and diabetes, hormones and thyroid disturbances, etc.) cannot be avoided.

It is with some such problems in mind that a movement is on foot to introduce a course in the third or fourth year, which will be essentially in the nature of a review of biochemistry from the point of view of the clinician, with biochemists and clinicians taking part in the seminars.

In the meantime, how do the medical (and dental) students react to biochemistry? In two instances, as a result of written opinions handed in anonymously, the students voted it the most popular in the school, even though they regarded it as one of the most difficult. Everywhere the students were encouraged to express their opinions—to criticize as well as to praise. Some of the objects of their criticisms were directed to lecturers who covered too much in too little time; to laboratory exercises which occasionally did not 'work'; and to textbooks which had too many pages ("if only we had the time to read all that stuff").

An Institute of Biochemistry. In the view of a number of biochemists (and others in related fields), "biochemistry has attained the status of an independent discipline." It can no longer be regarded as just a necessary appendage of the medical school; it belongs to the science department of the univeristy. Just as we have a department of chemistry, or of physics, so we should have a department of biochemistry—a department of the *university*.

It has been suggested that the model for such an arrangement is the Biochemical Institute at Cambridge, England, over which Hopkins presided for so long a period. This biochemical institute is the center toward which all biochemical activities are drawn. Here undergraduates, graduates and professional students are taught biochemistry. Here the staff is large and varied, and research activities are on the widest scale.

Of course, it is quite possible that the medical school may be some distance removed from the institute. In such a case, a subdivision of the department would be established at the medical school; but guidance would still largely come from headquarters—the central institute.

Such an idea, if carried out, would also avoid some duplications such as now exist at universities, where sometimes biochemical courses are given at various levels in various departments.

There is another aspect to this question. Most biochemical departments are attached to the medical schools. The authorities in charge of the school are primarily interested in the subject only in so far as it serves the medical student. They are not, as a rule, interested in the loftier aspirations of the biochemical staff: that of building up a graduate school and stimulating research activity. In fact, little of the funds supplied by the authorities to the biochemical department trickle to the graduate sub-division; and, indeed, were it not for the relative abundance, for the time being, of grants from government and private sources, the outlook for graduate teaching and research would be poor indeed. However, it is the hope of those urging the idea of an 'institute', or, at least, of establishing biochemistry on a university status, that appropriations for advanced work will come from the university itself, as well as from outside sources.

An Institute of Biological Research. At one university a separate institute has been established to pool the talents of men from industry and men from the university in solving some fundamental problems. Whatever is discovered is published. The institute is called a 'biological' one because it includes not only biochemists, but bacteriologists, endocrinologists, anatomists, ge-

neticists, physiologists, etc., drawn from the resources both of the university and industry; and each problem is attacked cooperatively from the broadest angle. Graduate students, working for their Ph.D., are the active assistants.

It is this emphasis on cooperation which is perhaps the characteristic feature of the institute. For example, a graduate student may be working on the problem of protein depletion in the diet. His immediate supervisor is the professor of biochemistry. But working with proteins and with animals, questions will arise which may need the attention of a specialist in protein chemistry, a pathologist, an endocrinologist, etc. These specialists are always available within the building to cooperate as actively as might be required.

Every program of research adopted by the Institute is the result of study by the members of its staff and its group of cooperative scientists, and the several sponsoring industrial, governmental and university laboratories. (This, incidentally, might hinder the productivity of a first-class mind, but it might encourage many a young man of talent to get the broadest foundation for the future.)

Two broad fields of interest that have engaged the attention of the Institute in recent years have dealt with the role and metabolism of proteins and protein derivatives in animals; and the origin, development and chemo-therapy of cancer.

CURRENT PRACTICES IN UNDERGRADUATE AND GRADUATE TRAINING OF BIOCHEMISTS

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If we look at the product of biochemical training in the United States, we find the quantity at the bachelor's level to be very small. Data collected by the Office of Education (*Higher Education*, vol. 12, page 56, December 1955), on earned degrees conferred by institutions of higher education, show 144 bachelor degrees in biochemistry for the year 1954–55, or .05% of the total degrees conferred. This means that relatively few institutions have an organized undergraduate major in biochemistry. In those institutions where the department of biochemistry is part of the medical school, and this includes about 75% of the total institutions, there would be no reason to have an undergraduate major. In the departments which are separate from the medical school, either in the college of agriculture or in the college of letters and science, or where the work is part of the chemistry department, a formalized major is usually available. This major consists of about 15 credits of work in biochemistry beyond an elementary course and includes two to four credits of thesis work. However, the number of students selecting this major is usually very low. For example, at Wisconsin the largest number taking such a major in one year was in 1924 when 15 students graduated with a major in biochemistry. In recent years the number at Wisconsin has been less than five. I indicated that the major was beyond an elementary course, since in the institutions where biochemistry is connected with agriculture, the department usually gives elementary courses for agricultural and home economics students. Often these courses include students in veterinary science and related areas. In medical schools such courses are offered for nurses and dental students, and often they are given without a formal course in organic chemistry as a prerequisite.

When we turn to the Ph.D. degree, we find quite a different situation, for in 1954–55 147 or 184 degrees were granted depending upon which figures are used. In any case there were more Ph.D. degrees than bachelor degrees. The only other fields in which I found this to be true were entomology and astronomy. In biology the number of Ph.D. degrees is 10–12% of the number of first degrees and in chemistry the figure is 20%. About 2% of the total Ph.D. degrees granted in a single year are given in biochemistry. For comparison we might indicate the following figures: biochemistry, 147; chemistry, 1,005; physics, 511; botany, 151; and zoology, 196. It is obvious,

therefore, that most of the graduate students in biochemistry do not enter with an undergraduate major in this specific field.

Although the bulletins of most graduate schools give as one of the entrance requirements that the student must have an undergraduate major in the field in which he wishes to work or the equivalent of such a major, it is obvious that the equivalent is used more often in biochemistry. From our own experience at Wisconsin, and from the comments in the letters which I received from chairmen of other departments, the students enter either with a background in chemistry or in biology. I have often remarked that our students are deficient either in physical chemistry or in biology. May I refer to two statements made in letters which I received. The first from the chairman of a department closely associated with the medical school, "Our general requirements for admission are training in biological subjects equivalent to that of pre-medical students and all the chemistry courses offered by the College. Admission is based mainly on personal interview at which an attempt is made to evaluate the knowledge and talent of the applicant. After admission, all of our special students are required to take advanced courses in organic chemistry and in physical chemistry, and during the first year here they take the course in biochemistry given in the first year of medicine." The second from the chairman of a chemistry department in which there is a division of biochemistry, "This means that all of our students have had one year of physics, one year of calculus, and a year each of general, analytical, organic and physical chemistry. They can be admitted as graduate students in biochemistry with no background in biology, and this is true of many of our students. One of our problems is to give such men an adequate background in biology."

After admission, I gather from the replies which I received, there is considerable emphasis on courses. Incidentally, some departments make a very careful selection, since one chairman said, "Last year we had 32 applications and admitted four students." Although there is a great variety of courses given under different names and with different number of credits, I imagine much of the same material is covered and by the end of the program a rather uniform training has been reached. When the biochemical work is given in a department of chemistry, there is a greater emphasis on chemistry. In the larger departments of biochemistry, a series of courses seem to be established including such subjects as proteins, carbohydrates, lipids, enzymes, vitamins, biochemical methods, plant biochemistry, cereal chemistry and dairy chemistry. One reply indicated that "Graduate students entering the department are about equally divided between those who have had an undergraduate course in biochemistry and those who have had none, the latter usually being chemistry graduates. In case they have had no experience in biochemistry, they are required to take our fundamental course without credit."

The same differences seem to carry over into the comprehensive examination. For example, from a department of chemistry which has a division of biochemistry all students who are candidates for advanced degrees are required to take the same qualifying examinations as those that are demanded of all Ph.D. candidates in chemistry. That is to say, he must pass with a grade of B, examinations in physical chemistry, organic chemistry, analytical chemistry and inorganic chemistry, each examination being of three hours' duration. In addition, the biochemist must pass a written qualifying examination in biochemistry. The chairman comments further, "I think it is highly probable that these requirements are to be changed. The change, if accepted by the faculty, will be in the direction of permitting him, if his interests are more on the biological side of things, to substitute qualifying examinations in, for example, physiology and microbiology for two out of the four stated examinations in chemistry."

The following is a comment from another department where the biochemical work is given in the chemistry department: "All of our biochemistry majors are required to take advanced courses in organic and physical chemistry, and in addition they have to pass comprehensive examinations at the end of their second year in these subjects. In addition, our biochemistry majors are required to pass a comprehensive examination in one other field of chemistry, i.e., analytical or inorganic." Another report gives the requirements in a modified form; "The department emphasizes the desirability of a broad training in chemistry and therefore requires a demonstration of some proficiency in the four areas of analytical, inorganic, organic and physical chemistry. Entering graduate students take written examinations in each of these areas. Failure in any of these examinations indicates a deficiency in that area. Such deficiencies must be

removed during the student's first year either by passing refresher courses or a second examination. In a typical year over two-thirds of our entering students will have at least one deficiency area and perhaps half of the students will have two or more." On the other hand we have the following description from a rather large department of biochemistry which is separate and distinct from the chemistry department: "A student must pass an oral qualifying examination before being admitted to candidacy for the Ph.D. degree. This examination is conducted by a committee appointed by the Graduate Council, usually selected from a list of persons suggested by the Graduate Adviser. The type of oral examination to be given the candidate is determined by the Committee selected by the Graduate Council, but it is the policy of the department to recommend that the proposition type of examination be used. In the proposition type of examination, the proposition should consist of short statements of original research problems, ideas, or theories, derived by the student from advanced courses, seminars and the biochemical literature. The student should be prepared to present the propositions in their proper historical background, give their significance, and be able to defend the propositions up to the limit of existing knowledge. Wherever possible, an experimental approach should be given for the solution of the proposition. The main stress should be placed on originality. The review or seminar type of report will not be allowed. The primary purpose of the examination is to test for the ability to apply fundamental principles of biochemistry to a research problem." I have given the outline of such an examination in some detail since I was interested to find that several departments now practice this procedure.

Studying both the letters received and the bulletins from various graduate schools, I was struck by the considerable emphasis on course work for the Ph.D. degree. Perhaps this is because I have often commented that graduate work depends upon a professor and a student. Of course, with increasing numbers of students it would be prohibitive for each student to learn all the techniques from the professor. Therefore, we have organized courses so that one professor may give training to several graduate students. This is quite acceptable if the courses are given to train the students for research rather than for the sake of saturating them with further facts and figures. In any case it is interesting that a very small proportion of the regulations dealing with graduate work in biochemistry is given to a discussion of research or thesis work. I imagine the reason for this is that the importance of research for the Ph.D. degree is taken for granted and that much of this information is transmitted directly by the major professor. Perhaps it might be well to restate some of the phrases given by President Gilman of Johns Hopkins during the early days of graduate work at that institution; for example, "The preparation of this thesis requires labor for the greater part of an academic year and the completed paper is supposed to show the candidates' mastery of his subject, his power of independent thought as well as of careful research, and his ability to express, in clear and systematic order, and in appropriate language the results of his study."

There seems to be considerable variation between institutions regarding the direction of the dissertation. In some cases it is clearly stated that a committee of three members must approve the thesis. In most other cases the work is directed by a single faculty member. There also seems to be considerable difference in the time of initiation of research work. Some clearly state that the dissertation is not started until the candidate is admitted for the Ph.D. degree. I found some suggestions regarding temporary faculty advisers and that "By the beginning of the third semester the student will be expected to have selected the faculty members with whom he will do his thesis problem." Others state, "As you might expect from this, our program is fairly heavy on course work, particularly in chemistry. To offset this we require our students to begin their research work as soon as possible after they enroll in the Graduate School." I know some do not agree with my point of view on this matter but I am going to state it nevertheless. The earlier, and by earlier I mean the first month, a graduate student comes in contact with a research problem, the better training this student will obtain. It is only when a student encounters actual experimental difficulties that a student will truly comprehend the literature in his field of specialty whether it be a detailed description of an analytical method or the attempt to explain certain results. You will note that I said come in contact with a research problem since I agree with Dr. C. N. H. Long[1] that if a student is assigned too soon to a particular problem, it is

[1] *Pediatrics* 15: 203, 1955.

likely that he will become too highly specialized in a single, often narrow, field.

I found no significant deviations in the language requirement. Many stated: "Students must have demonstrated a reading knowledge of German and French before they may be advanced to candidacy." In some cases the student may have a choice of two languages in which there is significant literature in his field.

Most of the outlines and statements give specific considerations to a minor. The minor most often mentioned is that of organic chemistry. Others include physical chemistry, bacteriology and physiology. More extensive work in the minor field is suggested by some departments; for example, "Two minor areas of study are required. The first must be outside of the Chemistry Department. The second minor is an area of chemistry." In this connection it is important to point out that biochemistry is frequently used as a minor area for other departments. In our basic course at Wisconsin, 'Principles of Biochemistry,' over one-half of the students enrolled are from related departments such as bacteriology, botany, zoology, plant pathology, etc.

A number of comments were made on the importance of seminars in graduate work. For example, "All students majoring in the department are obliged to register in our general seminar course, each student for the M.S. degree must take a minimum of three credits, and each student for the Ph.D. degree must take a minimum of six credits in this course." At Wisconsin we practice the same general procedure, that is all students must take a year of general seminar; beyond that we have individual journal clubs for various areas of work. Usually credit is not given for these journal clubs, but the credit is given through the research work. For example, the following comment comes from one of the chairmen: "Most of our staff members have more or less informal research seminars in addition to the departmental seminar."

A few chairman commented on the importance of the graduate students having teaching experience. However, I found no department where teaching experience was required of all students receiving the Ph.D. degree. It is apparent that a much larger proportion of graduate students are supported as research assistants than as teaching assistants. However, the importance of having some teaching experience cannot be underestimated. A survey of this situation in our department during the past several years indicates that well over half of all the Ph.D.'s which we turn out have had considerable teaching experience.

Perhaps we should say a few words about the master's degree in biochemistry. In some departments no attempt is made to provide graduate training beyond the master's degree. However, in other departments the students proceed either directly to the Ph.D. degree, or the master's degree is merely an intermediate in the total training for the Ph.D. degree. In one department it is stated: "In most instances students will be expected to enter the department as candidates for the M.S. degree. This will permit further evaluation and provide a terminal degree if it does not seem advisable to continue for the Ph.D. degree. Other students may, of course, only wish to devote the time required for the M.S. degree." It is my own feeling that the introduction of the master's degree is important for other reasons. Often the student is so busy gathering data that he fails to have much experience in preparing material for publication or for a thesis. Often the students attempt to prepare the final Ph.D. degree thesis in a short period of time and become frustrated in the attempt. If the student has had some experience in preparing a master's thesis, the later preparation of the Ph.D. thesis is much more effective. In an analysis that I made in 1949, 1950 and 1951 regarding the master's and Ph.D. degrees granted in the United States and Canada, I found about an equal number of the two degrees. However, in an analysis of the students receiving the master's degree only about one-half of them continued for graduate work. Thus it appears that about half of the students receiving their master's degree continue for a Ph.D. degree and about half of those receiving Ph.D. degrees do not stop for a master's degree.

In closing this paper I would like to make a few specific comments. I think it is obvious that in the field of biochemistry there is a close relationship between departments. It is apparent to me that where biochemistry is a division of the chemistry department, I always find emphasis of importance of working with related departments such as biology, bacteriology, and so on. In those cases where the Department of Biochemistry stands alone there is again great emphasis on the importance of working closely with the departments of basic chemistry. Since biochemistry is made up of two components, the bio part and the chemistry part, this relationship is especially significant. It has given us strong biochemists in

the past and I believe it is the only hope for the future. The relationship may be formalized by granting a joint degree between biochemistry and a related department. At Rutgers University a further attempt to bring biochemistry into closer contact with biology has been made through the Bureau of Biological Research. "The four departments of biology in the College of Arts and Sciences, Bacteriology, Botany, Physiology and Biochemistry, and Zoology divide the area of biology into its field of specialization. These departments nurture the development of the specialist and present in courses the fundamentals of each specialty. The Bureau of Biological Research is a fifth department where teachers and students from the other four departments may meet on common ground to study cooperatively problems in biology. A graduate student, for example, may elect to specialize in biochemistry. If so, he will be trained in the fundamentals of this specialty in the Department of Physiology and Biochemistry. The student's major professor, as a member of the Bureau as well as the Department of Physiology and Biochemistry, may bring him into the integrating environment of the Bureau for his experience in research and in working with other specialists. In this environment, the student will find a group of specialists with their students who will discuss his problems with him, examine his experimental designs, his results, and all of his ideas critically and sympathetically. Thus both student and teacher, even though they are specifically trained as biochemists, are forced to see their work and thoughts from the viewpoints of the physiologist, pathologist, endocrinologist, nutritionist and all the other specialties. They learn to see the living system in its true dimensions and not as an unreal image which reflects the limitations of the specialized artist."

Another point which struck me in a few comments was the importance of special equipment and service facilities. For example, one report stated these service facilities include the microanalysis, infrared, ultraviolet spectra facilities, ultracentrifuge, mass spectrometer, electrophoresis equipment, and so on. Biochemistry has relied in the past on the availability of animals of various species, of plants and of microorganisms, but I am convinced that in the future we must also supply the physical instruments needed for accurate measurements.

This brings us to the methods of training students in the use and understanding of these instruments. Several replies referred to courses on biochemical methods or biochemical techniques. Personally I am worried about courses of this kind because they seem to take a tremendous amount of time from the student. However, in our own department I can honestly say that Dr. Strong has developed a course in this area which is tremendously valuable to our students and does not require an extraordinary amount of time. At the University of Chicago arrangements are made for students to work with two members of the staff—each for half of one quarter—on a specific problem assigned by the staff members.

If you will allow me I should like to mention one innovation which we have established in our department known as the departmental seminar. As the department grew and individual professors or groups of professors established their own journal clubs we were beginning to appreciate that graduate students could be trained in our department without knowing what was going on in other areas. Our departmental seminar meets once a month and every student who receives a Ph.D. degree in our department must appear before this group. All of our graduate students and all our staff members attend these meetings. The student, therefore, has the experience of presenting his material before a fairly large audience. The student also knows that this is a critical audience and that questions may be put to him from many different individuals. It also gives the entire department an opportunity to know what is going on, what progress is being made in various areas and how this progress may relate to their own individual work. While graduate work will undoubtedly remain in the realm of a relationship between a professor and a student, the problem of communication will become ever more difficult. Thus, whatever methods we can use not only for communication but for the improvement of communication will undoubtedly help in a field such as biochemistry which does cut across several disciplines.

In conclusion, I think it is obvious that each of the some 60 departments of biochemistry use different techniques for the training of graduate students. I hope that this situation will continue and that no attempt will be made to standardize the methods. Only one standard needs to be used by all departments and that is a quality product. The methods of reaching the end product may be many and diverse.

PLANNING A COURSE IN MEDICAL BIOCHEMISTRY IN A NEW SCHOOL: REUNION WITH PHYSIOLOGY

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THE ALBERT EINSTEIN COLLEGE of Medicine of Yeshiva University is being developed on the basis of two concepts: utilization of programs which experience has established as sound, and experimentation in newer areas or programs of medical education, with sufficient objectivity and flexibility to permit subsequent alterations in the curriculum, as experience may indicate. One educational experiment presently in progress in the Albert Einstein College of Medicine is the teaching of the first year courses in biochemistry and physiology as a single discipline. This is the first year of operation of the College of Medicine and consequently the data should be considered preliminary and certainly incomplete. The present first year class consists of 55 students; beginning in September 1956, each entering class will be 96 in number.

Before this experiment is described, several obvious and generally known postulates inherent in all experiments in medical education should be restated. These are given in what is our idea of the order of their importance. First, the faculty should have enthusiasm for and interest in a proposed new program and should be qualified for its development. In terms of integration and correlation of subject matter, this implies that faculty members themselves will have a significant degree of interest in, and knowledge of, those associated areas to which their own field of special interest is being related. As a minimum, the interests of the participating faculty should be of adequate breadth to permit education of the staff as well as of the students. Second, a soundly conceived and thoroughly discussed program. Third, good interpersonal relations between faculty and students. These relations are influenced greatly by the overall philosophy of a school with respect to attitudes toward students, including interest and enthusiasm of faculty for the education of the student, and the intellectual atmosphere which is provided for the student. Fourth, the nature of the physical facilities, which can have a great influence on the ease with which a new program may be developed.

Let us now consider the experiment under discussion, namely, planning a course in biochemistry in a new medical school: reunion with physiology. The term reunion recognizes that biochemistry and physiology were once as one. Up to the beginning of the 19th century, knowledge from studies of living systems was not segmented or classified as to whether the contribution was made by physiologists, chemists, or so-called chemical physiologists or physiological chemists. As the body of scientific knowledge regarding living organisms grew, and as an increasing number of diversified tools from various sciences were applied to the fundamental problem of the nature of living things, specialization inevitably developed. The expansion of scientific knowledge encourages specialization. Physiology arose as a branch of anatomy; biochemistry as a branch of physiology and chemistry, and, more recently, of physics. Pathology had its origins in anatomy and physiology, and pharmacology in chemistry and physiology. This increasing specialization was, and continues at the present time, logically to be encouraged by specialized mentors, each with his own particular 'school' of training. Each of these basic sciences, in their application to medicine, has focused on the fundamental problems of the nature, functions and mechanisms of actions of living things, in order to understand more completely the aberrations or derangements which occur in disease, so that more logical preventive and therapeutic measures may be instituted.

The subject matter of biochemistry has been developed from studies of the composition of cells and the mechanisms by which the isolated components of cells participate in cellular phenomena. The biochemist has attempted to relate these events to the intact animal. In turn,

the knowledge of physiology has derived from studies of the whole animal, of organs, and of organ systems. The data obtained by the biochemist from *in vitro* studies have been utilized by the physiologist to explain *in vivo* phenomena. Like the biochemist, who has studied cellular phenomena *in vitro* as well as *in vivo* by the use of classical physiological tools, the physiologist has often derived for himself information from *in vitro* systems examined through chemical analysis. In the case of some systems it is not possible, and is indeed futile to separate information into biochemical and physiological compartments. Both disciplines have the common aim of explaining physiological phenomena in physical and chemical terms. This is apparent whether one is examining the distribution of ions or solutes across cell membranes, the mechanics and chemistry of respiration, kidney function, or the activity of the nervous system.

For educational goals, whether these be in the medical curriculum or in the research laboratory, lines of demarcation between biochemistry and physiology are becoming increasingly dim. One can discern both groups of specialists converging to a greater and greater degree on a more specifically circumscribed area of interest, the living cell. The organ and organ system physiologist is considering the cellular phenomena of the organ under study; the biochemist, long engaged in taking cells apart and isolating components in purified or crystalline form, is becoming increasingly concerned with the reconstruction of the component parts into a functioning unit. We are, indeed, witnessing a renaissance of cellular biology, in which period our identity as biochemists and as physiologists may well disappear.

It is recognized that separation of biochemistry and physiology continues to be useful for purposes of administration and for making specialization possible, both with respect to the teaching of specialized aspects of each subject and the training of specialists. The identification and correct classification of the scientists involved will not be lost, but will continue to stem from the nature of their published researches and from the type of training given in their laboratory to future teachers and investigators.

When an analysis of the subject matter of biochemistry and physiology is made, four broad areas are apparent. These are:

1) The nature and quantity of the constituents of living things.

2) The nature and mechanisms of the reactions in which the constituents of living organisms participate.

3) The physiological manifestations of these reactions.

4) The mechanisms by which the diverse activities of the organism are integrated to produce unification of the human body as a single functional entity.

With this information an approach has been made to the formulation of a single, unified course in biochemistry and physiology. This objective was sought in every aspect of the teaching, namely, laboratory, conferences and lectures, with the view of eliminating reference to whether a particular topic under discussion was biochemistry or physiology. However, to facilitate the program, and pending further education of the faculty of the two departments, major responsibility for various portions of the course has been allotted to those faculty members initially most familiar with that material. As experience and knowledge are acquired, this aspect of the program may be gradually decreased.

The teaching program has been based on a philosophy in which the major degree of emphasis is placed on laboratory work, on the student doing things with his own hands, and with most of the teaching being done with small groups of students, in the laboratory and in informal conference groups. Review questions are provided and discussed. Didactic lectures are reduced in number. The lecturer elucidates material difficult of interpretation in textbooks, presents recent developments, and provides a rounded picture of a subject by bringing together related material from diverse fields. In general the lectures do not

TABLE 1

I.	Physical-chemical principles
II.	Cellular composition
III.	Enzymes
IV.	Biological oxidations
V.	Respiration
VI.	Energy metabolism
VII.	Carbohydrate metabolism
VIII.	Muscle
IX.	Circulation
X.	Protein metabolism
XI.	Lipid metabolism
XII.	Nutrition
XIII.	Large intestine; intestinal movements; feces
XIV.	Kidney: water and electrolyte metabolism
XV.	Special tissues and special body fluids
XVI.	Special senses

XUM

TABLE 2. DISTRIBUTION OF HOURS IN BIOCHEMISTRY—PHYSIOLOGY COURSE

Type of Exercise	Hr.	% of Total Hr.
Lectures	36	9.5
Laboratory	252	65.0
Conferences	62	16.0
Clinical application of basic sciences	36	9.5
Totals	386	100.0

repeat material which the student can readily read. Examinations in course are given to aid the student in assessing his own progress, and to help the faculty to ascertain which students may need extra instruction. Grades are occasionally recorded by the faculty and sometimes are not; policy on this point is still undecided.

Initially, the faculties of the two departments each prepared a list of topics which, on the basis of their past experience, seemed desirable to include in a teaching program for the first year medical students who would come prepared with previous instruction in gross anatomy, histology, embryology, genetics and some neuroanatomy. The list of topics was discussed at great length and subsequently arranged for presentation in the order indicated in table 1. It should be noted that neurophysiology and endocrinology, traditional segments of the instruction in physiology and/or biochemistry, are missing from the subject list. In the present curriculum of the Albert Einstein College of Medicine, neurophysiology and neuroanatomy have been organized as two separate but parallel disciplines presented by the Departments of Physiology and Anatomy, and endocrinology as a single course organized by the Departments of Anatomy, Biochemistry and Physiology. Table 2 shows the relative distribution of hours among lectures, laboratory and conference sessions in the course presently in progress. The hours designated as 'Clinical Application of the Basic Sciences' are devoted to currently studied concepts illustrated by clinical problems either in the form of patient presentation or as a symposium organized around a particular topic, with participation by clinical faculty. These hours are also used to show films. Evaluation of experience will undoubtedly be followed by modifications of the teaching program. As mentioned previously, the hours provided for neurophysiology and endocrinology are not included in the course under discussion, but are encompassed in other courses. These hours

TABLE 3. TOTAL HOUR DISTRIBUTION IN BIOCHEMISTRY–PHYSIOLOGY, INCLUDING NEUROPHYSIOLOGY AND ENDOCRINOLOGY

	Biochem.-Physiol. (Table 2)		Neurophysiol.		Endocrinol.		Totals	
	Hr.	% of total hr.	Hr.	% of total hr.	Hr.	% of total hr.	Hr.	% of total hr.
Lectures	36	9.5	14	17.0	6	13.6	56	10.9
Laboratory	252	65.0	44	53.6	27	61.4	323	63.1
Conferences	62	16.0	12	14.7	6	13.6	80	15.6
Clinical application of basic sciences	36	9.5	12	14.7	5	11.4	53	10.4
Totals	386	100.0	82	100.0	44	100.0	512	100.0

are indicated in table 3 in order to complete the picture for the reader who may wish to make comparisons with other first-year curricula. In general it would appear that the total hours represented in table 3 by biochemistry-physiology, neurophysiology and endocrinology are within the range of the total number of hours allotted to biochemistry and physiology together in medical schools generally. The present program differs largely in the relative distribution of these hours among the various types of teaching exercises.

The laboratory work emphasizes teaching in small groups, maximum student participation, quantitative concepts, modern tools, and logical development, wherever possible, of experiments from preceding exercises. Two full days per week are set aside for laboratory work, and some experiments require more than a single laboratory day. Thus certain aspects of the laboratory instruction may be eligible for designation as project studies or project programs.

The student laboratories were constructed to accommodate eight students each, and were designed to permit the teaching of most laboratory exercises commonly conducted in courses of biochemistry or physiology. The floor plan of a typical laboratory is indicated in figure 1. There are twelve of these unit laboratories, to accommodate an anticipated maximum class of 96 students; each unit laboratory is equipped as a research laboratory with the students sharing all of the equipment in the room. In addition, there are six laboratory rooms for use in experiments with special equipment which cannot be duplicated in every student laboratory. One might immediately infer that division of the class into

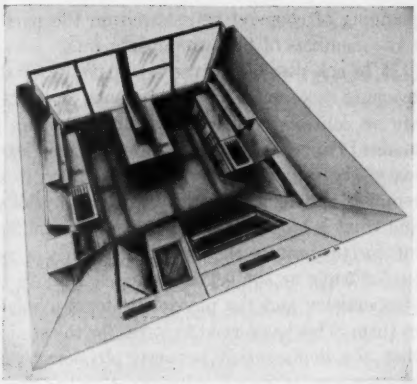

FIG. 1. Perspective drawing of a unit student teaching laboratory designed for use by a group of eight students in Biochemistry–Physiology.

groups of eight, in separate laboratories, may entail a need for a significantly larger staff, in comparison with the requirements of the traditional larger student laboratories for the basic sciences. However, this need not be the case, provided the teaching staff exhibits a degree of enthusiasm with respect to moving about among rooms. Thus, a given number of instructors in the usual single large laboratory generally walk around the laboratory in order to work with various students. If in such a large laboratory one now creates a few partitions and connecting doors, the availability of the instructor to the student becomes dependent on the teacher's willingness to go through doors into adjacent rooms, rather than taking his customary stroll from area to area in a large laboratory.

In experiments which utilize special equipment, e.g. Warburg apparatus, paper electrophoresis, etc., the students work in the special laboratory rooms in groups, on a rotation basis. This means that several different experiments may be under way on a single laboratory day, and that this group of experiments may be repeated over a period of several laboratory days, in order to permit all students eventually to participate in each experiment.

In planning the laboratory experiments, the Physiology Department members reviewed the experiments proposed by the biochemists and made suggestions regarding how the biochemical principles involved in various *in vitro* experiments could be illustrated equally well in the intact animal. Similarly, the biochemists found it possible in some instances to suggest quantitative biochemical measurements or parameters for the assessing of physiological functions. It is obvious to us that much further growth in these directions can be expected in subsequent years as each group becomes more aware of the other's field. The list of laboratory experiments finally planned for the current year is presented in table 4. The experiments are listed in the order of their scheduling for the class. Certain of the experiments are exercises which are conducted concurrently by different laboratory groups, and represent a sequence of experiments through which all students ultimately rotate during a period of several weeks. It should be mentioned that some experiments are done completely by each student, whereas others are conducted on a cooperative basis, involving 2, 4 or 8 students working as a team.

What evaluation can be presented at this time of the experiment of teaching biochemistry and physiology for medical students as a single

TABLE 4. BIOCHEMISTRY-PHYSIOLOGY: LABORATORY EXPERIMENTS

Experiment	Experiment	Experiment
1. Osmotic behavior: red cell	11. Rebreathing	21. Heart-lung preparation
2. Metabolism of red cell in relation to osmotic changes	12. Breath holding	22. Biosynthesis of proteins *in vivo*
3. Body fluid compartments	13. Oxidative phosphorylation	23. Biosynthesis of heme *in vitro*
4. Sicklemic hemoglobin	14. *p*-Aminohippurate transport *in vivo* and *in vitro*	24. Life span of the erythrocyte
5. Electrophoresis	15. Carbohydrate absorption and glycogenesis	25. Nutrition:
6. Enzymes	16. Harden-Young effect	a. Nutritional anemia (rat)
7. Biological oxidations	17. Muscle	b. Thiamine deficiency (pigeon)
8. Lung pump	18. Gastrointestinal movements	c. Ascorbic acid deficiency (guinea pig)
9. Alveolar ventilation and dead space ventilation	19. Biogenesis from acetate *in vivo*	d. Pyridoxine deficiency (rat)
10. Oxygen dissociation of hemoglobin *in vivo*	20. Circulation	e. Choline deficiency (rat)
		26. Kidney: water and electrolyte metabolism

course? At best the objective assessment of the degree of success of any experiment in medical education is exceedingly difficult if not impossible. Innovations or deviations from what is customary are both new and untested. Except for a similar effort at the University of Western Ontario, we are not aware of other current attempts to present biochemistry and physiology as a single subject. The present experiment, like others in medical education, can be described, but not precisely evaluated. Moreover, in this instance the experience and data available are much too short and few. Indeed, it is not unlikely that this will be a continuing experiment, with new experiences and information being obtained during each successive year of the course. It is only possible at this time to record a few preliminary impressions.

First, it is our opinion that the faculty of the two departments have benefited greatly by the program attempted. The enthusiasm of the staff members possibly stems in part from the newness of the laboratory experiments, and the attendant expansion of knowledge of each teacher who is participating. The members of the Department of Biochemistry are relearning the physiology which may have been studied as a course sometime between 5–25 years ago. In turn, the physiologists are coming again in contact with basic biochemical principles, to which each had formerly been exposed. This has not just been the experience of reviewing a subject once taken as a requisite for a higher degree, but also having emphasized for one the vast extent to which knowledge has expanded in the intervening years. In addition, relationships between biochemistry and physiology not discernible at an earlier stage of intellectual development suddenly assume reality and greater significance. This has led to planning of cooperative research on the part of some members of the two departments.

It is not possible to assess the value of this teaching experiment for the students, since there are no controls with which comparisons can be made. It appears that the student, in his learning of the subject matter, is not troubled to attempt to distinguish between what is physiology and what is biochemistry. The students are finding for themselves that not infrequently a particular topic or subject is discussed in both the biochemistry and the physiology texts available to them. This has served to stress for the student that the demarcation between physiology and biochemistry is, in many instances, either dim or arbitrary.

It is the opinion of the two departments that emphasis on extensive reading and on work in the laboratory has encouraged significantly the intellectual growth and independence of most of the students. Of the present student body of 55, 15 students are planning to conduct research work during the coming summer vacation. Periodic written exercises and frequent conferences have provided evidence that the students are acquiring those fundamental principles of biochemistry and physiology which, in the opinion of the faculty, will provide each student with the basic information necessary from these subjects for a career in the practice of medicine or in teaching and research. Perhaps this experiment will provide further evidence that arrangement of subject matter is less important than the ability and enthusiasm of the faculty. It is possible also that the experiment in progress may support the concept that while knowledge of constituent parts of a curriculum is essential, awareness of interrelationships among these parts will facilitate their understanding by, and increase their significance for, the student of medicine.

THE TEACHING OF BIOCHEMISTRY IN AN INTEGRATED MEDICAL CURRICULUM

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A MAJOR EXPERIMENT in the methods of medical education is being conducted at Western Reserve University. It has involved a curriculum revision which crosses all department lines and becomes effective from the student's first day in the medical school until his graduation. While integration of the subject material is an important part of the new curriculum, this by no means covers the entire scope or purpose of the revision. The changes go beyond methods of presentation of facts. One of the most unique and important features of the change is that plans have been made by the combined efforts of the entire faculty of the medical school.

Before discussing the role of biochemistry in the new curriculum, it is necessary to describe the curriculum in some detail. The revised program of medical education was started in 1952 and the first students are graduating this year. During these four years final plans often have been completed just a step ahead of the oncoming class and during three of these years both the old and the new types of curriculum were being taught simultaneously as students in the old curriculum completed their training. While some parts of the new curriculum have been given for four years, others are now being given for the first time. Although considerable experience has been gained in teaching biochemistry in this curriculum, clearly there is much experimentation yet to be done before anything resembling a stable structure is obtained. The curriculum will be modified over the years as data are obtained from which critical changes can be planned.

The general plans were made as follows: it was agreed by the faculty that there would be three phases in the medical curriculum and these are indicated in figure 1. *Phase 1*, lasting one year, concerns normal structure, function, growth and development. *Phase 2*, lasting one and one half years, concerns alterations of normal structure, function, growth and development and study of disease. *Phase 3*, lasting one and one half years, concerns the clinical application of the material learned in *Phases 1* and *2*. In order to formulate the first broad plans of the three phases, three interlocking committees were set up, one for each phase, with each department represented on the three committees. By faculty decision these three committees were instructed to plan the teaching as a cooperative venture of the faculty on an interdepartmental basis with organized teaching groups to carry out particular portions of the program. Therefore the broad plans were set up by the three committees and the curriculum of each phase was divided into subjects as illustrated for *Phase 1* in figure 2. Then subject committees were appointed with the interdepartmental representation shown in figure 2, and these subject committees made the final detailed plans and did the actual teaching.

The study of the normal biology at a cellular level is the first subject. The subject committee on cell biology includes the study of development of cellular energy, cellular growth and cellular structure. The properties of enzymes and an introduction to intermediary metabolism are given. The material is presented in a correlated manner by three biochemists, a histologist, a physiologist, two microbiologists and a representative of the clinical department. Biochemistry plays a major role in this subject committee but by no means the exclusive role. Microbiology makes up part of this subject quite naturally since some of the best examples of cellular growth and metabolism come from microbiology.

The next subject committee is on tissue biology and the neuromuscular system. In this committee the anatomy and physiology, along with the histology and the biochemistry, of the locomotor and the nervous systems are considered. Anatomy plays the dominant role in this committee. Gross anatomy is limited to approximately 65 hours in *Phase 1*. Dissection in *Phase 1* is done on the infant cadaver. The major part of gross anatomy and dissecting of the adult cadaver is postponed

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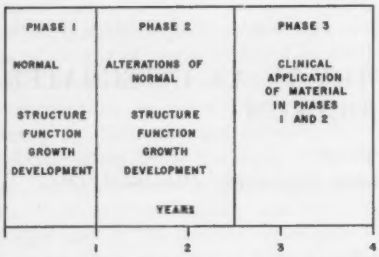

FIG. 1. The three phases of the four-year medical curriculum.

to the second and third years so that it may be correlated with diseases and with examination of patients. In *Phase 1*, the neuromuscular system is followed by consideration of the cardiovascular system and the respiratory system in which physiology takes the leading role. Next the metabolism subject committee covers the digestive system, the liver and kidney. Here again biochemistry plays a substantial role. The last committee considers the endocrine system and it includes the reproductive system.

Each subject committee has a representative from at least one clinical department. The subject committees present correlation conferences using patients to illustrate the importance of the basic science which is being presented.

To improve over-all correlation there is a coordinator for each of the three phases. All subject committees submit their plans to the coordinator of the phase. The coordinator goes over the entire plan with all the chairmen and co-chairmen of the subject committees of the respective phases. The completed plan is submitted to the heads of the departments that are involved for approval of subject matter and of teaching personnel. The actual teaching is under the direction of each subject-committee chairman once the plans have been approved and the departments as such play a minor role.

Examinations have been greatly curtailed.

	ANATOMY	BIOCHEM.	PHYSIOL.	OTHERS
CELL BIOLOGY	1	3	1	3
TISSUE BIOLOGY AND NEURO-MUSCULAR	4	1	2	2
CARDIOVASCULAR AND RESPIRATORY	2	1	2	1
METABOLISM	2	2	3	1
ENDOCRINES	2	1	4	1

FIG. 2. Staff of the subject committees of *Phase 1*.

Each subject committee gives an examination but the examination is given anonymously and the grades are not recorded. The answers are graded either 'satisfactory' or 'unsatisfactory' and returned to the student. In this way the student can evaluate his performance and the subject committees can evaluate what they have accomplished. A comprehensive examination is given at the end of each phase, which again is graded 'satisfactory' or 'unsatisfactory' and only the unsatisfactory students are identified. These examinations replace the continual series of examinations which were given at Western Reserve University; there were 30 or more in a year and departments frequently vied for the student's time by way of the examinations.

An important part of the interdepartmental teaching is the multidiscipline laboratory in which the students carry out experiments designed by the subject committees. The *Phase 1* laboratory is pictured in figure 3. In addition to the laboratory benches, it contains writing desks which are used for microscopy and for study. This laboratory is the student's home base. It is open at all times and his space is not used by any other student. Anatomy, physiology, histology, biochemistry and microbiology are all done in this laboratory. There are three such laboratories, one for *Phase 1* and two for *Phase 2* since the latter covers parts of two years. These laboratories replace the seven departmental laboratories of the old curriculum. The gross anatomy in *Phase 1* is done in the multidiscipline laboratory, but the adult cadaver is dissected in *Phase 2* in the department of anatomy. In *Phase 1* the laboratory consists of six units such as pictured and each houses 16 students. These small laboratory units lead to quiet, more serious work and allow intimate supervision by the staff. Laboratory experiments have been designed which require integration of the disciplines and illustration of principles. Routine tests for this or that compound have been eliminated unless the test is actually required in an experiment. Correlation with the didactic material is stressed.

The above discussion presents a very brief picture of the subject committees' activity in *Phase 1*, but this is only one of the four activities as shown in figure 4.

Subject Material. The subject committee teaching requires a major part of the time and provides the basic floor of facts and principles. By the subject committee's approach, integration is encouraged and there is careful screening of

material for selection of the most important items of study. Since complete coverage is impossible, basic principles are emphasized and non-essential material is eliminated. There is a great deal of give and take in the subject committees.

Clinical Methods. The clinical material of *Phase 1* deals with the patient-physician relationship. Each student is assigned a normal pregnant mother who has been carefully selected by a social worker from patients registered at the University Hospitals. The student visits the home, follows the mother's course, observes the delivery and then studies the growth and development of the infant. Integrated with the observation of the mother and child are didactic and discussion groups. Supervising this clinical program is a physician who as preceptor meets with his group of eight students once or twice a week. The student has four different clinical preceptors with whom he works in the three phases of the curriculum. The long and continued contact with the different physicians does much to help the students in acquiring good habits of medical practice learned through example and leadership.

Scientific Method. The third major activity of *Phase 1* is the project. The project is designed to stimulate learning of the scientific method through participation in a scientific investigation. During the second half of the first and second years one day each week has been set aside in both *Phases 1* and *2* for the student to work on his project. This is a required part of the curriculum. The staff of both the clinical and preclinical departments submit projects. The students select their preference or submit suggestions for a project of their own design. In the latter case, they are required to find a faculty sponsor. A project committee screens all projects to determine whether they are suitable. Primarily this involves a decision of whether the project will illustrate the scientific method and whether it is too difficult for the time available. The projects may consist of laboratory experiments, library research or a study of clinical or other subjects. Projects are designed to bring experience in depth, critical evaluation and participation in methods of investigation and verification. A written progress report is required at the end of the first and second years. The student may continue the project in his third and fourth years. A thesis on the subject is required in the fourth year. The project provides a long-term contact of faculty and student in which the student can learn by example. Projects are graded as 'satisfactory' or 'unsatisfactory' and form part of the student's record. During the summer vacation many students elected to work on their projects and they may obtain summer—research fellowships.

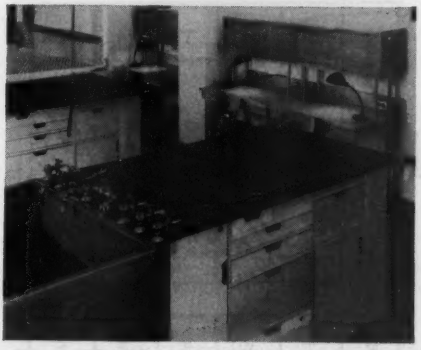

FIG. 3. Multidiscipline laboratory of *Phase 1*, for 16 students in which the student does all his laboratory work for the first year. The bench in the foreground accommodates two students; writing desks are for microscopy and study.

Self-Education. Free time and elective time are provided to encourage the student to carry on much of his own education. In the previous curriculum in the School of Medicine the student had little chance to develop his own initiative since he attended classes 5.5 days a week. There was little time to do anything else. On those occasions when there was free time it was an hour here and an hour there. In the present curriculum during the first 2½ years the student is given 1½ days of free time in the middle of each week in which he has the initiative to choose his own activities. There is an elective program in *Phase 3* which is an extension of the philosophy of giving the student the responsibility of conducting his

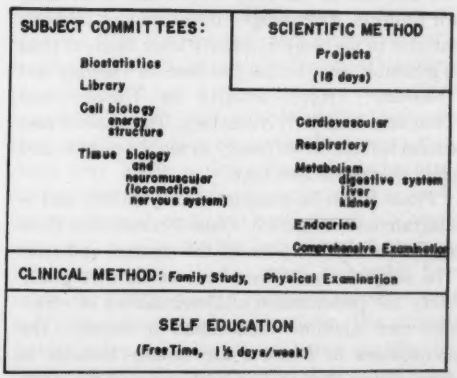

FIG. 4. Diagram of *Phase 1*, the first year, showing the subjects in the two semesters (9 months).

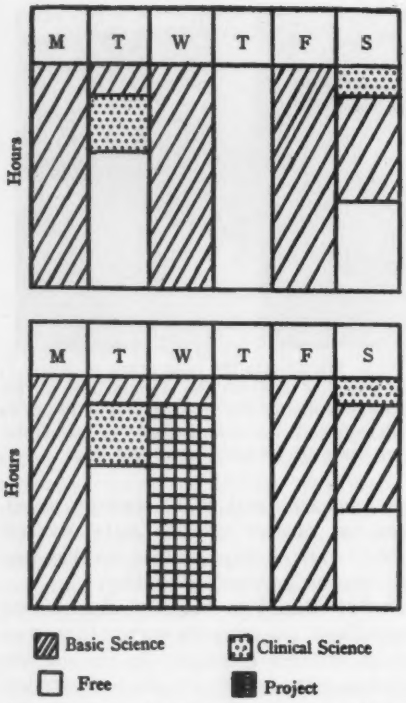

FIG. 5. Daily schedule of time in first and second semesters of *Phase 1*.

own education in a manner which is left to the individual.

Figure 5 gives an outline of the daily schedule for the student in *Phase 1*. In the first half of the year there are three activities: subject committee teaching; clinical science for two hours on Tuesday and one hour on Saturday; and free time on Tuesday afternoon and all day Thursday. During the last half of the year Wednesday is set aside for projects. Accordingly, if the student takes an interest in his project, a fairly large block of time is available since he has free time on Tuesday and Thursday. Project activity on Tuesday and Thursday is entirely voluntary. The student also makes his call on his family to see the mother and child during his free time.

Phase 2 can be considered only briefly and is diagrammed in figure 6. *Phase 2* constitutes three semesters and is broken by the summer vacation. The subject committees have as their responsibility the presentation of abnormalities of structure and function which make up disease. The committees in *Phase 2* are usually broader in membership than in *Phase 1* because of the addition of members of the several clinical departments. Disease is presented from many aspects including etiology, mechanism of injury, symptoms, clinical features, diagnosis and treatment, and these are considered as an integrated whole. To obtain this integration, all the specialities of the clinical and basic science fields contribute.

The student is introduced to disease as related to cells and tissues, then to infectious diseases and chemical agents. The latter study includes general pharmacological principles. Then he considers diseases of the organ systems and finally interrelated disturbances of several systems. It is during the presentation of organ systems that the dissection of the adult cadaver is done. The anatomy of each system is studied as the disease of that system is considered. Detailed neuroanatomy and dissection of the head and neck are taught in the third year.

The projects and free time are continued in *Phase 2*. The clinical study of the mother and baby is expanded to include the siblings.

We will say little about *Phase 3*. An overall schedule of the three phases is shown in figure 7. There are three required clerkships in *Phase 3*. The first is a clerkship of four months in which the principles of clinical medicine are taught in a cooperative fashion without primary concern for any branch of medicine. The problem of the patient is the integrating factor and the clerkship is conducted by an interdepartmental committee from medicine, surgery and other clinical fields. The other required clerkships are two months in obstetrics and gynecology and two months in ambulatory medicine. There are six months for elective time in which the student may elect other clerkships, courses (clinical or preclinical), projects or other special problems including courses or work in another university.

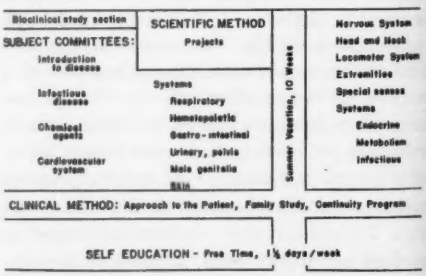

FIG. 6. Diagram of *Phase 2*, the second year and the first half of the third year, showing the subjects in the three semesters (14 months).

In *Phase 3* bioclinical study sections are introduced. These study sections are designed for groups of 10–15 students. They give the opportunity to study a selected disease in detail, and to gain more experience in a selected department. This study is designed to illustrate the contribution of both biological and clinical sciences in the study and elucidation of disease. Each group of students is under the supervision of a faculty team, one member from a basic science department and one member from a clinical science department. The students have the choice of bioclinical study sections offered by the different departments. Each section is conducted during 16 half days in a given semester and the student takes two such bioclinical study sections. These study sections provide an opportunity for study in depth. They show the student that much that he is taught in subject committees is superficial and that the problem is more complex and often less clearly understood than it appears in the survey presentations, which subject committees are forced to give due to the limited time they have available. Bioclinical study sections bring the clinical and preclinical staffs together. They serve to continue basic science teaching in the clinical years.

In the usual medical curriculum there frequently is a large gap in understanding between the preclinical and clinical sciences. The present program is aimed at a better understanding between the two groups with the thought that each should supplement the other throughout the four years. Although the emphasis changes throughout the curriculum, as shown in figure 8 the preclinical and clinical teaching is presented at all times.

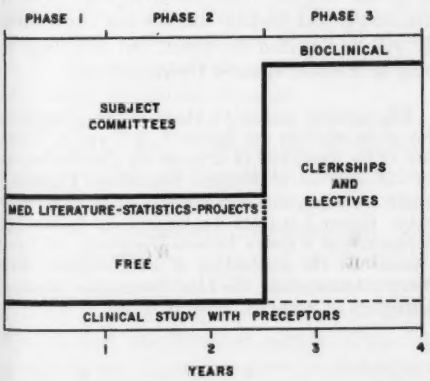

FIG. 7. Diagram of the three phases of the four-year curriculum.

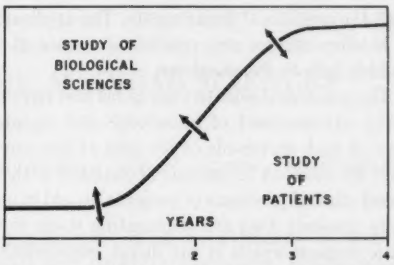

FIG. 8. Distribution of time between clinical and preclinical studies in 4 years and cross-over of disciplines.

There is a natural interrelation of ideas in the teaching of subject committees, in correlation conferences, in projects and in bioclinical study sections.

It is seen that an attempt is made in the curriculum to promote four major types of learning: *1*) a floor of facts and principles which are essential tools for the physician; *2*) an experience in the scientific method in the belief that the scientific method is part of a good physician's armamentarium; *3*) self-education since it is necessary that the physician continue his education throughout his life; *4*) proper attitudes toward himself as a physician and toward medicine as a profession.

One criticism of the above discussion may be that it describes in a general way the curriculum but does little toward telling about the teaching of biochemistry. One of the first questions frequently asked is, "Do the students learn as much biochemistry with this method as they did in the old curriculum?" Also it is asked, "Is this a good method of teaching biochemistry?" The answer to the last question is that this is a method of presenting an integrated course in medical science and is not intended to be the best method of presenting biochemistry. The answer to the first question, "Do they learn as much biochemistry?" is difficult. The students of the former curriculum probably could pass a more detailed examination immediately after they completed the biochemistry course, but over the years the present students may retain a larger fund of biochemical information. In the present curriculum they have longer and more continued contact with biochemistry, not because the biochemistry staff teaches much more in the clinical years, but rather because our clinical faculty strives to correlate and apply biochemistry in their teaching. The clinical faculty is becoming better and better qualified to do this because they are associated more and more

with the preclinical departments. The application of biochemistry or any preclinical science at the bedside falls to the physician.

The preclinical scientist has as his first responsibility advancement of knowledge and maintenance of high standards of his field at his university. To attempt to become acquainted with the broad clinical problems of patients would in most cases preclude deep and penetrating study in the basic science which is his direct responsibility. Not only is the preclinical faculty responsible for training medical students, they are responsible for training Ph.D. graduate students and for training post doctorals (both Ph.D. and M.D.). To do this they must provide leadership in their speciality. The staff of the biochemistry department limits most of its teaching to *Phase 1* and to a few subjects in *Phase 2*. Biochemical subjects are taught by biochemists and they do not attempt to teach anatomy, histology, etc. Nevertheless, through reading in a limited subject, there is opportunity to become acquainted with what the other members of the committee are presenting and to integrate. If the laboratory is anatomy, the anatomy department provides staff for its supervision; if the experiment requires special experience in physiology, the physiology department assumes responsibility. However, in many instances the laboratory has an integrated theme and has been so designed. In such experiments the interdepartmental subject committee conducts the laboratory. If the full power of a university faculty is drawn upon, as can be easily done in subject committee teaching, where men from all departments are used, it is usually possible to find a person who is fully competent to assist in teaching the biochemistry at any level of *Phase 1*, *Phase 2* or *Phase 3*. The bioclinical study sections in *Phase 3* are set up in a field of interest to the individuals presenting the subject and thus offer little problem.

The actual teaching of biochemistry is no more time-consuming or difficult in the present curriculum than in former curriculum. The planning and evaluating takes time; the devising of new laboratory experiments is costly, but such experiments should be devised in any curriculum. Project teaching is expensive of faculty time, but this is not an essential part of the subject committee teaching. Projects can and have been conducted with departmental type teaching (Yale).

The great advantage of the curriculum at Western Reserve University is that it permits the large teaching staff of the medical school to consider medical teaching as a single body. Yet the system preserves the department structure in which people of similar interests unite to foster their field and to provide advanced training for the Ph.D. At Western Reserve University the preclinical departments are just as interested in the training of Ph.D.'s as they are in training the M.D. The Medical School looks on the training of Ph.D.'s as part of its function in a university. *Phase 1* serves as the basic course for our graduate students covering the requirements in biochemistry, physiology, histology, anatomy and microbiology. Projects and clinical science are not taken by the graduate students. *Phase 1* is a prerequisite for all advanced courses in biochemistry, microbiology and physiology, and for the biochemistry degree advanced courses in chemistry are required. Most important is extensive research in the laboratory on the subject of the Ph.D. thesis. A staff active in research, with critical judgment, enthusiasm for their field and eager to offer advice and aid to those they will train is essential for a good Ph.D. program. The new medical curriculum does not interfere with the fostering of such a staff either by increased time required for teaching nor by demands placed on the preclinical faculty in the clinical teaching.

It is too early to evaluate the success of the program, but my observation is that most of the faculty and students like the new curriculum. It will be modified no doubt, but it is here to stay at Western Reserve University.

The author wishes to thank J. W. Patterson for permission to use figures 1, 2, 7 and 8. These are to be published in a paper by Dr. Patterson in the *Journal of Medical Education*. Figures 4 and 6 are a personal communication from T. Hale Ham. Figure 5 is from *An Experiment in Medical Education at Western Reserve University. A Symposium on the Evaluation of the Program*. New York: Association of Life Insurance Medical Directors of America, 1954, p. 3.

GRADUATE TRAINING IN THE MEDICAL SCIENCES

ERIC G. BALL

From the Division of Medical Sciences, Harvard Medical School, Boston, Massachusetts

IT IS MY MAIN PURPOSE today to describe for you our experiences with a special course which has been established at the Harvard Medical School for the primary purpose of training graduate students in the basic medical sciences. Before I do so, it will be best if I first outline briefly the framework within which our graduate training program is carried out.

All candidates for the M.A. or Ph.D. degree in the departments of anatomy, bacteriology, biological chemistry, pathology, pharmacology or physiology at the Harvard Medical School are enrolled in the Division of Medical Sciences of the Graduate School of Arts and Sciences. This Division is an administrative device through which these six basic medical science departments are able to funnel their relations with the Graduate School of the Faculty of Arts and Sciences as a group instead of separately. The Division of Medical Sciences is thus a unit of the Faculty of Arts and Sciences and is housed in the Medical School. Its teaching staff is comprised entirely of members of the Faculty of Medicine and a certain selected number of these are chosen each year to sit with the Faculty of Arts and Sciences as representatives of the Division. Thus there is achieved a marriage of convenience, and, I may add, a happy one, which permits the Medical School faculty to participate in the recommendation of the M.A. and Ph.D. degrees to students enrolled in the Graduate School of Arts and Sciences.

Such an arrangement has not only simplified the administrative management of graduate training in the Medical School, but has also served to unify the approach to graduate training in the basic medical sciences without, however, curtailing the individual rights of the separate departments. Until four years ago, the training program of a graduate student in the Division had the following pattern. Upon admission he was required to name the department in which he desired to obtain his degree. This department then planned the student's schedule. By agreement of all departments, it was required that all Ph.D. candidates, besides taking course work in their major subject, must also pass with an honor grade course work in two collateral fields. For example, the candidate for the Ph.D. in biochemistry would take the biological chemistry course for medical students to satisfy the requirements for his major subject. As collaterals he could present two of the other basic medical sciences, for example physiology and bacteriology, here again taking the courses given to the medical students in order to fulfill these requirements. Or he might present instead as his collateral courses advanced physical and organic chemistry, journeying to Cambridge to take these courses. This type of program was flexible, but it had many drawbacks. First, it presented problems in course scheduling for the entering student and often left him with a makeshift and weak schedule for his all important introductory first half year since, for example, biological chemistry and physiology were not offered until the second semester. Second, a student in biochemistry could graduate under such a program with little or no concept of the contributions being made to medical science in the fields of pharmacology, bacteriology, histology or, even in a few cases, in physiology. Finally, the formal course training he did receive in the basic medical sciences was obtained as a member of a class of 130 medical students. Without being too derogatory, let it suffice to say that such training was certainly not ideal for developing that core of tough intellectual processes needed for individuals destined for a career of research and teaching.

This dissatisfaction with the graduate training program led us, some years ago, to explore ways and means for improving it. After much deliberation in committee meetings, it was decided to establish a special full-year course which would serve to give basic training to all first-year graduate students regardless of the field in which they chose to major. With the generous aid of the Commonwealth Fund and the hearty

support of Dean Berry and the heads of the various departments, this course was launched in the fall of 1952. The broad objectives of this course were to be the following:

1. To introduce the student to the literature, experimental methods and fundamental principles associated with each of the basic medical sciences.
2. To present this material in an integrated fashion in such a way that the course centers around the morphological and functional characteristics of organs and organ systems rather than around the discipline.
3. To stress thoroughness of approach to the topics selected rather than to attempt too wide a coverage.
4. To endeavor to inculcate in the student an ability to evaluate data critically and to present orally experimental results in a clear and concise manner.

The course is now in its fourth year and I believe it is fair to say that it has in a large measure lived up to these objectives.

Let me now briefly outline some of the main features of this course.

Schedule. The course is scheduled for five days a week for the full academic year. It constitutes the entire program of the first-year graduate student. A lecture is given each morning at 9 o'clock which lasts for an hour to an hour and a half, and it is immediately followed by a discussion period in which students and staff join. The remainder of the day is devoted to work in the laboratory or library, or is made free to to student to use as he sees fit. No rigid laboratory schedule is adhered to, the program being molded to the needs of the particular task at hand. Thus, the time factor is not allowed to be a deterrent to the undertaking of any project. The schedule is shaped to fit the experiment and not the experiment to fit scheduled hours.

Curriculum. Previous descriptions of the course (1–4) have dealt with the details of the curriculum. It is sufficient to say here that the course begins with the single cell as its topic and deals with its structure, chemical composition, metabolism and function, proceeds to the consideration of multicellular units and organ systems, and concludes with the treatment of the whole animal and its relationship to the environment. An effort is made to present topics in logical sequence and each topic is developed in such a way as to permit an integrated treatment of the contributions made to it by the various basic medical sciences. For example, the kidney is dealt with after the alimentary tract, lungs, vascular system and heart have been covered; then there are studied within a period of two to three weeks the embryological and structural features of the kidney, the processes of urine formation, the influence of drugs on renal function and renal hypertension. The combined experimental techniques of anatomy, physiology, biochemistry, pharmacology and pathology are thus brought to bear on the study of the kidney as a functional unit.

It should be stated that gross anatomy is not covered to anywhere near the extent that it is in the regular course for medical students. Only those aspects of gross anatomy are presented which are necessary to permit an understanding of the function of the organ system under study or of the relationship of this organ system to the animal as a whole.

The Staff. The staff is composed of one member from each of the departments of anatomy, bacteriology, biological chemistry, physiology, pharmacology and pathology. Each department head designates the member of his department who is to participate in the course. It is arranged for this member to be relieved of other teaching duties and only those are chosen who have a real desire to participate in the program. Staff members are rotated. In the four years the course has been running, each department has had at least two representatives and some three. Such rotation of the staff serves a double purpose. First, it permits more staff members to savor the course and really to understand its purpose. Second, it continually introduces new ideas and view points. As chairman of the course, it has been interesting for me to watch the growth of enthusiasm of new staff members as they begin to taste the full flavor of this approach to teaching. Until one has bitten deeply into it for himself, this flavor cannot be appreciated, nor once tasted can it be adequately described to others. It is like love; all the poets' descriptions of it are no substitute for the experience.

Another interesting aspect of the course as it relates to the staff members is the education that they themselves receive. The instructor not only renews and extends his acquaintance with the subject matter of fields other than his own, but also comes to see more clearly the interrelationship between his research approach and that

of his colleagues. Indeed, the course has been the stimulus to the launching of more than one joint research project by its staff members.

The course also profits by the voluntary participation of other members of the Medical School staff. During the early phases of the course we have the willing and helpful cooperation of members of the late Dr. Cohn's department in presenting the physical chemical aspects of the cell. From time to time, other faculty members give lectures or participate extensively in the planning and supervision of certain laboratory experiments. This contact with faculty members has in some cases so aroused students' interest that it has led them, on completion of the course, to pursue their thesis work in the particular area presented by these faculty members.

The Laboratory. A special laboratory has been constructed to accommodate 20 students. The floor plan of this laboratory has already been published (3). It is designed and equipped so as to permit all types of experimental work to be performed and also to give the student a home base where he can study if he so desires. It contains a small reference library. Each student is given a key to the laboratory and building and he is free to come and go at any hour of the day and night. We have found that this home laboratory stimulates and facilitates the exchange of ideas among the students. As Henry Steele Commager has recently written in his article entitled "The Problem Isn't Bricks—It's Brains" (5), "Given the right atmosphere, the appropriate facilities, and time, students will educate each other."

The laboratory experiments are for the most part newly designed. They are a credit to the ingenuity and enthusiasm of the staff. The mere amalgamation and assembling in juxtaposition of the laboratory experiments employed in the various medical student classes has certainly had no place in this course. For most of the experimental work the class is divided into groups, optimally of four members. Each team works on a different aspect of the topic under study, performing a thoroughgoing job with repetition of experiments when needed. The complete exercise may require a week or two for each group to complete. At the end of the block of time assigned to the topic, each group works up the data it has obtained and presents them to the class and staff at a conference period. Such conference sessions may consume a whole day. Each group's results and conclusions are critically evaluated by the class as a whole and discussed in relation to the literature and lectures. I must be frank to admit that the laboratory conference sessions early in each course are discouraging and sometimes disjointed affairs. As the year progresses, however, their character changes markedly and they become exciting and stimulating discussion groups. These conference sessions, to my way of thinking, provide a far better means for the evaluation of students than any examination system yet devised. It is here that one sees most clearly the growth and development of the students' minds. The ripened fruit of these conferences is evident at the end of the year when we hold an oral examination at which each student defends, before the staff and invited examiners, some proposition of his own formulation previously screened by the staff. The titles of some of these are arresting, as can be seen from the following few examples:

1) Is stereochemical specificity an obligatory condition of biological processes?

2) Acetylcholine functions as a synaptic transmitter in the central nervous system.

3) The invading bacteriophage provides the gene component responsible for the altered metabolism of the bacterial cell.

4) Immunological paralysis, actively acquired tolerance, and the tumor-enhancing effect have at their basis a common mechanism.

5) Enzyme adaptation involves protein synthesis.

Upon completion of the course, the student enters the department of his choice to complete his training and carry on his thesis work. Students are not required to make this choice until they have completed the course. It is of interest to note that to date nearly one fourth of the students have selected a field different from that which they designated as their goal upon entrance. Bacteriology and pharmacology gain at the expense of biological chemistry. Since over half of all students enrolled enter biological chemistry anyhow, this transfer of allegiance is a healthy one from the standpoint of medical sciences as a whole.

Another unexpected dividend provided by the course is the difference in attitude of the students who have completed the course to the taking of further course work. There is no longer any need to encourage, indeed I should perhaps say force,

students to take certain advanced courses without which they will find themselves handicapped in their careers. The need for training in certain areas in which they are deficient has become abundantly clear to them and they are eager to rectify such deficiencies.

The course has also served to attract a larger and better qualified group of candidates for admission. Most of our current applicants specifically mention the course as one of their reasons for applying at Harvard. The objects and design of the course thus seem to appeal to students, and the interest of students is aroused before they enter. It is therefore obvious that if any evaluation of the merits of the course are to be attempted, they will be complicated by the fact that we are undoubtedly dealing with better raw material than under the former system.

During the last three years a handful of medical students has also been enrolled in the course. A group of students was selected from the top portion of the entering medical class and offered the opportunity to substitute this course for the usual first-year curriculum. This was done not with the intent of luring these men away from medicine into the pre-clinical sciences, but to obtain some knowledge as to whether a course of this type is practical and feasible as a training program for medical students. My own conclusion would be that this type of program is indeed practical. Whether it provides a better basic training for the physician than the customary program cannot be answered by our experience to date. It must be recognized that the men we have selected are of such high caliber that they would undoubtedly thrive under any program. I would, however, venture to say that these men have profited by their experience. It is certainly true that they have enjoyed it. These medical students take gross anatomy in their second year, using time gained by reason of their study of certain aspects of pharmacology, pathology and bacteriology in this special course.

Now to conclude my remarks you are perhaps expecting me to make some evaluation of our experiences. You might ask, for example, how does the student trained in this special course compare with those trained by the former system, or will the student so trained become a better teacher or research worker? I doubt that such questions can be answered now or even in the future. Certainly any judgment that I might attempt to give would be prejudiced because I must frankly admit I am extremely enthusiastic about this type of training program for our graduate students. None the less, there is no denying that the course has certain distinct advantages and I have already dealt with some of these. However, it is not these points that I would like to stress now. The most important thing to emphasize, I believe, is the fact that the course has provided a means whereby the keen interest and enthusiasm of both teachers and students have been aroused. The exact design, curriculum and schedule of this course, or any course for that matter, are secondary in my opinion to the question, "Does it provide the proper stimulus to teachers and students?" Nothing dulls interest and enthusiasm so much as to "ladle out knowledge from a stagnant pool," as Joel Hildebrand phrases it (6). I have concluded from my experience with the young and enthusiastic group of teachers who have participated in this venture with me that we need have no fear for the future of education in the medical sciences. We need but give our younger generation of teachers a little encouragement and support, permit them to cast off the shackles of tradition, and we shall truly achieve the solution to some of our more pressing educational problems.

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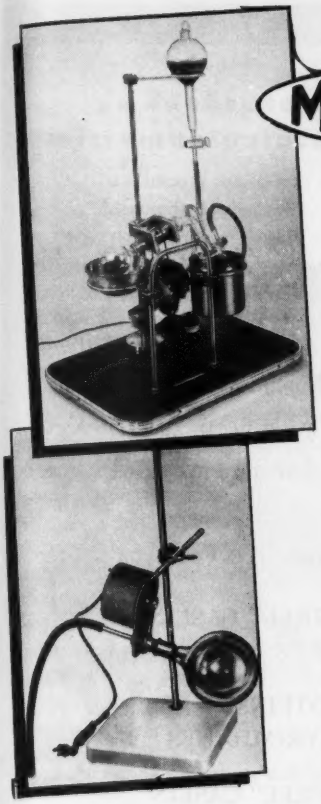

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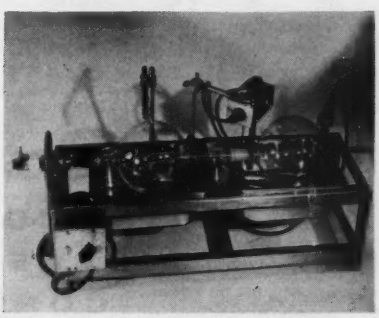

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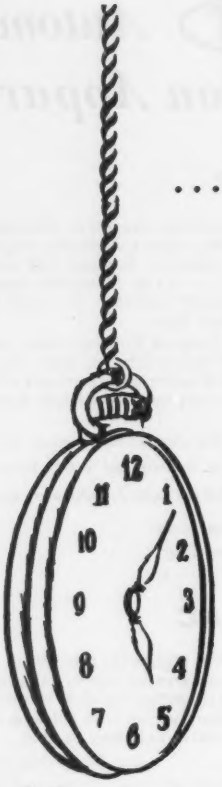

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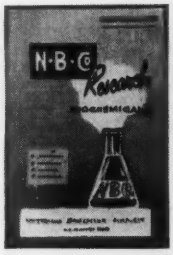

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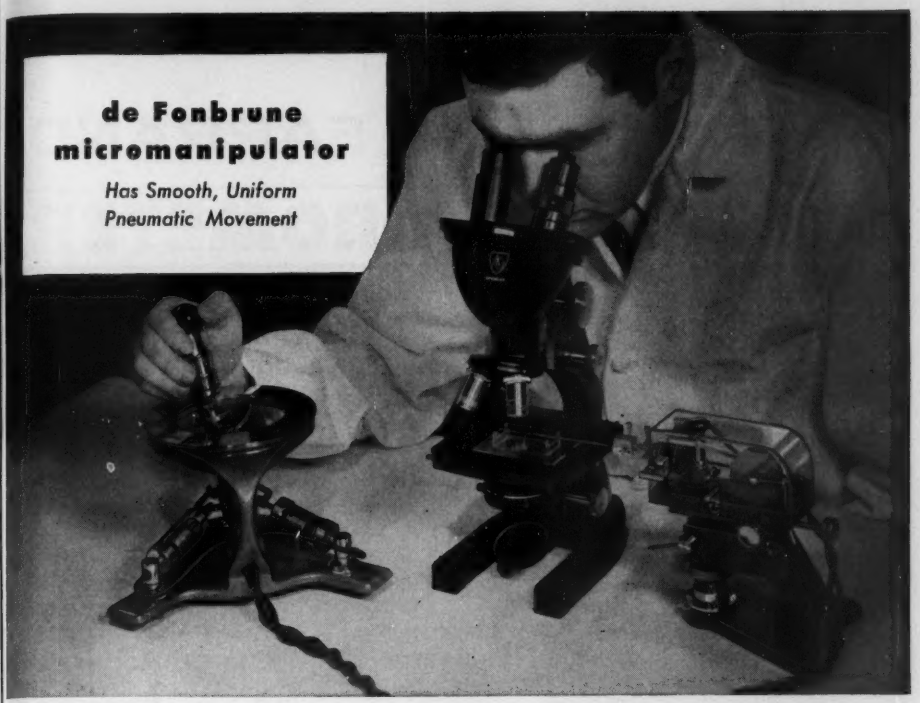

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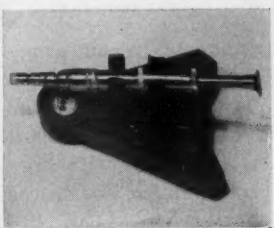

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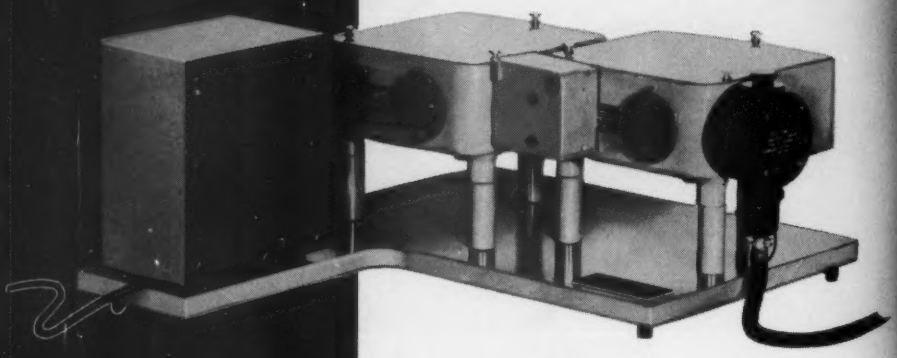

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* *Spectrophotofluorometric Assay in the visible and ultra-violet — R. L. Bowman, P. A. Caulfield, S. Udenfriend, Science, Vol. 122, No. 3157, July 1955.*

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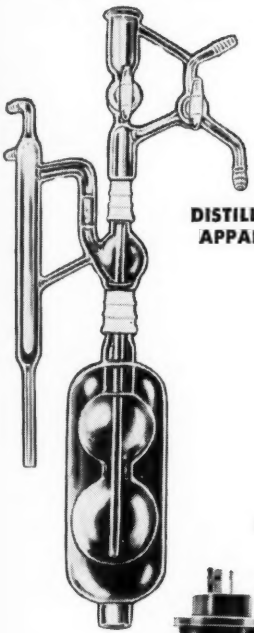

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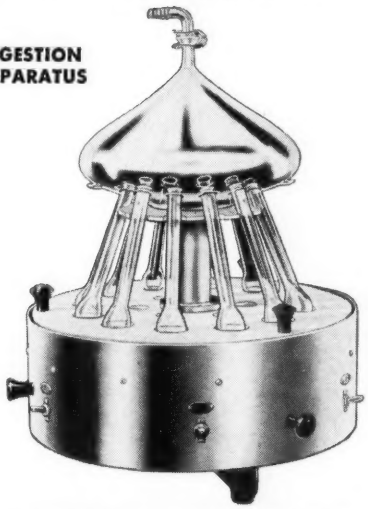

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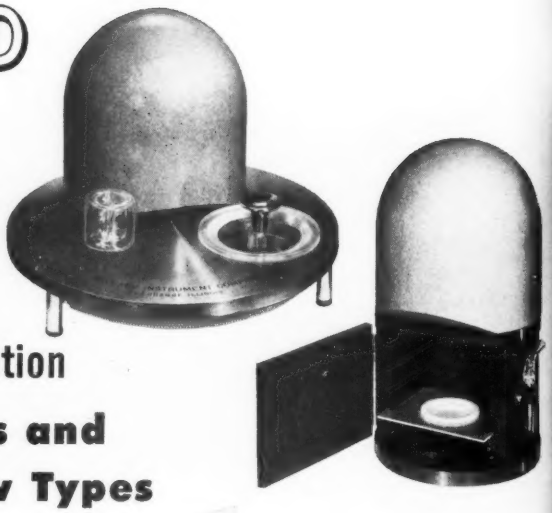

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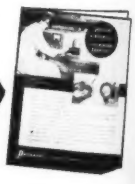

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